Video Article

Particles without a Box: Brush-first Synthesis of Photodegradable PEG Star Polymers under Ambient Conditions

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Abstract

Convenient methods for the rapid, parallel synthesis of diversely functionalized nanoparticles will enable discovery of novel formulations for drug delivery, biological imaging, and supported catalysis. In this report, we demonstrate parallel synthesis of brush-arm star polymer (BASP) nanoparticles by the "brush-first" method. In this method, a norbornene-terminated poly(ethylene glycol) (PEG) macromonomer (PEG-MM) is first polymerized via ring-opening metathesis polymerization (ROMP) to generate a living brush macroinitiator. Aliquots of this initiator stock solution are added to vials that contain varied amounts of a photodegradable bis-norbornene crosslinker. Exposure to crosslinker initiates a series of kinetically-controlled brush+brush and star+star coupling reactions that ultimately yields BASPs with cores comprised of the crosslinker and coronas comprised of PEG. The final BASP size depends on the amount of crosslinker added. We carry out the synthesis of three BASPs on the benchtop with no special precautions to remove air and moisture. The samples are characterized by gel permeation chromatography (GPC); results agreed closely with our previous report that utilized inert (glovebox) conditions. Key practical features, advantages, and potential disadvantages of the brush-first method are discussed.

Video Link

The video component of this article can be found at https://www.jove.com/video/50874/

Introduction

Polymeric nanoparticles have been widely studied for their potential use as platforms for drug delivery, supported catalysis, biological imaging, and self-assembly[1-3]. Modern applications require that nanoparticle syntheses be facile, reproducible, compatible with chemical functionalities, and amenable to diversification[4,5]. Ring-opening metathesis polymerization (ROMP) of strained olefins is a powerful methodology for the synthesis of functional polymeric nanostructures with controlled sizes and narrow mass distributions[1,6-8]. For example, norbornene-functionalized poly(ethylene glycol) (PEG) macromonomers (MMs) can be efficiently polymerized via ROMP to generate water soluble bottle-brush polymers. Using this approach, nanostructures that carry multiple releasable drug molecules, fluorophores, and spin-contrast agents can be prepared rapidly and in parallel[6,9,10].

ROMP has also been used for the "arm-first" synthesis of star polymers. In the arm-first method, linear polymers are crosslinked with a multi-functional crosslinker to give spherical nanostructures with polymeric arms. Schrock and co-workers reported the first arm-first ROMP synthesis of star polymers via crosslinking of norbornene, dicarbomethoxynorbornadiene, and trimethylsilyl protected dicarboxynorbornene linear polymers with a bifunctional norbornene crosslinker.[11,12] Buchmeiser has extended this methodology for the synthesis of materials with a range of applications that include supported catalysis, tissue-engineering, and chromatography[13-17]. Otani and coworkers have made star polymer nanoparticles with functional surfaces via a related "in-out" polymerization strategy[18,19].

Most arm-first polymerizations involve a complex interplay of monomer, polymer, and star coupling reactions. The latter proceeds via a step-growth mechanism that typically leads to broad molecular weight (MW) distributions. To overcome this limitation in related arm-first atom transfer radical polymerization reactions, Matyjaszewski and coworkers performed arm-first crosslinking of preformed polymeric MMs to provide star polymers with very narrow MW distributions[20]. In this case, the steric bulk of the MMs, and the increased ratio of star arms to initiation sites, inhibited poorly controlled star+star coupling processes, and led to a living, chain growth mechanism.

When we attempted the same strategy in the context of ROMP with a norbornene-terminated PEG-MM and a bis-norbornene crosslinker, star polymers with very broad, multi-modal MW distributions were obtained. This result suggested that in this system the MM alone was not sufficiently bulky to inhibit star+star coupling. To increase the steric bulk of the star arms, and potentially limit this uncontrolled coupling, we attempted to first polymerize the MM to form bottle-brush polymers in the absence of crosslinker and then add the crosslinker. We were pleased to find that under certain conditions, this "brush-first" method provided straightforward access to "brush-arm star polymers" (BASPs) with narrow MW distributions and tunable core and corona functionalities.

We recently reported the brush-first ROMP synthesis of PEG BASPs using Grubbs 3rd generation catalyst ***A*** (**Figure 1**)[21]. In this work, exposure of PEG-MM ***B*** to catalyst ***A*** generated a living brush macroinitiator with defined backbone length (***B1***, **Figure 1**). Transfer of aliquots of the ***B1*** to vials that contained different amounts of crosslinker ***C*** initiated BASP formation. The MW, and therefore the size, of the BASPs increased geometrically with the amount of ***C*** added. We provided a mechanistic hypothesis for this geometric growth process and demonstrated that functional, nitroxide core- and corona-labeled BASPs could be readily prepared without the need for post-polymerization modification steps or sequential monomer additions. However, in all of the reported examples, we were concerned about catalyst deactivation; we carried out all reactions under N_2 atmosphere inside a glovebox.

Since our initial report, we have found that the brush-first method is very effective for the formation of BASPs from a wide range of norbornene-terminated MMs and functional crosslinkers. We have also discovered that the method can be performed on the benchtop with no special precautions to remove air or moisture.

Herein, a series of three BASPs of differing MWs will be synthesized by the brush-first method under ambient conditions. In brief, 10 equivalents of ***B*** will be exposed to 1.0 equivalents of catalyst ***A*** (**Figure 1a**) for 15 min to yield a ***BI*** with an average degree of polymerization (DP) of 10. Three aliquots of this batch of ***BI*** will be transferred to separate vials that contain 10, 15, and 20 equivalents (*N*, **Figure 1b**) of ***C***. After 4 hr, the polymerizations will be quenched via addition of ethyl vinyl ether. The star polymer MWs and MW distributions will be characterized using a gel permeation chromatography instrument equipped with a multi-angle laser light scattering detector (GPC-MALLS).

Protocol

We first describe the synthesis and purification of PEG-MM ***B*** from 3 kDa O-(2-aminoethyl)polyethylene glycol (PEG-NH_2) and norbornene-*N*-hydroxysuccinimidyl (NHS) ester. The former compound can be purchased from Sigma Aldrich Inc., or prepared via anionic polymerization according to literature procedures[22,23]. The latter compound can be prepared in two steps according to a published procedure[21]. Next we describe a synthesis of catalyst ***A*** from commercially available Grubbs 2nd generation catalyst. We then demonstrate the use of this complex for brush-first BASP synthesis. This experiment details the procedure for making BASPs with *N* = 10, 15, and 20 from a ***BI*** with DP = 10. All reactions were performed in a fume hood using standard scintillation vials.

CAUTION: Always wear gloves, a lab-coat, and lab glasses, and follow common laboratory safety practices when working with hazardous chemicals. Any organic solvent must be handled in a fume hood. Solids can be weighed out on a balance outside the fume hood. Chemicals should not come into contact with skin, eyes, or mouth. It is strongly recommended to read the MSDS for every solvent and solid used in this procedure before beginning.

1. Preparation of PEG-MM *B*

1. Add PEG-NH_2 (300 mg, 0.0001 mol, 1.0 eq) to a 40 ml scintillation vial equipped with a stir bar.
2. Dissolve the PEG-NH_2 in 3 ml of anhydrous *N,N*-dimethylformamide (DMF).
3. Add 36 mg of norbornene-NHS ester (0.000105 mol, 1.05 eq)[21].
4. Cap the vial and stir the reaction mixture overnight at room temperature.
5. Remove the stir bar and add diethyl ether to the reaction solution to precipitate the PEG-MM ***B***.
6. Filter the white fluffy precipitate and wash extensively with diethyl ether. Alternatively, transfer the suspension to a 50 m centrifuge tube, centrifuge at 4,000 rpm for 5 min at room temperature, and then decant the supernatant. Add fresh diethyl ether, centrifuge, and decant again. We recommend repeating this procedure 3x for a total of 5x.
7. Dry the precipitate under vacuum for 24 hr to remove residual diethyl ether.

2. Purification of PEG-MM

In our previous report, the PEG-MM ***B*** was prepared from commercially available PEG-NH_2 and was used for BASP synthesis without further purification after drying (*i.e.*, after step 1.7). In this study, we vary the PEG-NH_2 source (commercial versus homemade), and we compare BASP formation results before and after more rigorous preparative high performance liquid chromatography (prep-HPLC) MM purification. In the remainder of this study, the dried MM obtained after step 1.7 is referred to as ***B1***. Prep-HPLC was used to purify ***B1*** to give ***B2***. An analogous prep-HPLC purified MM synthesized in our laboratory via anionic polymerization is referred to as ***B3***. Prep-HPLC was performed using a Beckmann Coulter HPLC (127p solvent module and 166p detector module) with a 1-ml sample loop and an Agilent Zorbax 300SB-C18 PrepHT reverse-phase column at room temperature.

1. Set-up HPLC with solvent A: deionized water (Millipore purification system, 18.2 Ω) with 1% acetic acid; solvent B: acetonitrile.
2. Prime pumps and equilibrate column with 95% A and 5% B.
3. Dissolve PEG-MM in acetonitrile or MeOH (150 mg/ml).
4. Filter through a 13 mm 0.45 µm Nylon syringe filter.
5. Set HPLC method:
   - Flow rate: 20 ml/min
   - 0-1 min: linear gradient to 10% B and 90%A
   - 1-10 min: linear gradient to 90% B and 10% A
   - 10-13 min: switch to initial conditions (5% B and 95%) and re-equilibrate column
   - Set UV detector to detect absorbance at 256 nm
6. Load 0.8 ml of sample onto the sample loop.
7. Inject sample.
8. Collect the major absorbance peak (under the conditions specified, the product elutes between 5-7 min).
9. Repeat as necessary. Combine pure fractions together in a round-bottom flask.

10. Remove all solvent via rotary evaporation.
11. Redissolve the product in dichloromethane and add sodium sulfate. Gently shake or stir the flask periodically for # 1 hr.
12. Filter the mixture using a fritted glass filter.
13. Concentrate via rotary evaporation. Dry under vacuum overnight.
14. The PEG-MM can be characterized by ^{1}H-NMR in CD_2Cl_2 (15-20 mg/0.7 ml CD_2Cl_2, 500 MHz or higher is recommended with over 128 scans and relaxation delay, d1 = 2.0 sec), and MALDI-TOF using positive ionization mode and 2-(4-hydroxyphenylazo)benzoic acid as the MALDI matrix.
15. The PEG-MM can be stored for months in a scintillation vial at 4 °C.

3. Preparation of Catalyst A

1. Add Grubbs 2nd generation catalyst (500 mg, 0.589 mmol) to a 20 ml vial equipped with a stir bar.
2. Add pyridine (approximately 0.474 ml, 5.89 mmol, 10 eq) to the vial. The solution color should immediately change from red to green. Allow the reaction to stir until all of the red color has disappeared and the solution has become viscous (15-30 min).
3. Fill the reaction vial with cold pentane to precipitate complex ***A***.
4. Filter the suspension to collect the green precipitate (catalyst ***A***). Wash 4x with 15 ml of cold pentane.
5. Dry the green solid under vacuum overnight.
6. Complex ***A*** can be stored for months at room temperature in a benchtop desiccator without significant loss of activity. For extra precaution, we typically store the complex in a -20 °C freezer inside a glovebox. For convenience, we pre-weigh known amounts of ***A*** into 4 ml scintillation vials immediately after drying (step 3.5). We then store these vials in the glovebox freezer. When ready to run a ROMP reaction, we simply take one vial out of the glovebox and use as described below (step 4.4).

4. Preparation of Stock Solution of Living Brush Polymer (*BI*) with DP = 10

1. In a 3 ml vial with a gas-tight screw cap equipped with a stir bar, weigh out 65 mg (0.020 mmol, 10 eq) of MM ***B***. This amount corresponds to 20 mg of MM for each of the 3 different sizes of BASPs, and 5 mg leftover for GPC analysis of the ***BI***. Use a spatula to add the MM directly to the bottom of the vial. Try to prevent material from adhering to the sides of the vial as this scenario could lead to MM contamination in the final BASP product.
2. Dissolve the MM ***B*** in 158 µl of THF. Immediately cap the vial after adding THF to avoid solvent evaporation. Note: The final concentration of MM during the polymerization should be 0.05 M. If 158 µl of THF is added here, then 243 µl of catalyst solution, step 4.4, will be added to give 401 µl of THF total, which corresponds to [MM] = 0.05 M. The amount of solvent during this step can be varied, as long as the amount of solvent during step 4.4 is also varied to give [MM] 0.05. We have found that polymerizations carried out with [MM] < 0.05 sometimes do not proceed to complete conversion.
3. Let the solution stir until all of the MM is dissolved. Heat lightly if necessary. Avoid splattering the viscous solution onto the sides or the cap of the vial.
4. Next, add a known amount (2.8 mg for this example) of catalyst ***A*** to a 3 ml vial (or obtain a vial with pre-weighed catalyst ***A***). Add anhydrous THF (466 µl in this example) to give a 6 mg/ml catalyst solution. Cap the vial immediately. Allow the catalyst to completely dissolve; gently shake the vial if needed. *This catalyst solution should be used immediately for ROMP*. Note 1: The catalyst solution should be a forest green color. If it is black, or greenish-brown, then it has likely decomposed, and it will probably not yield satisfactory ROMP results. If decomposition occurs, we suggest preparing fresh catalyst (according to section 3 above), or using freshly distilled THF. Note 2: The amount of THF added to ***A*** is chosen to ensure that the final [MM] is ~0.05. This amount can be adjusted, as long as compensatory adjustments are made to the MM solution in step 4.2.
5. Add 243 µl (1.46 mg; 1 eq to ***B***) of the catalyst solution quickly (not dropwise) to the stirring MM solution. Try to keep the needle tip just above the MM solution when adding to the vial. Avoid splashing the reaction mixture onto the sides and cap of the vial, as splashing can lead to residual MM and ***BI*** impurity in the final BASP.
6. Cap the vial immediately and let the reaction mixture stir for 15 min to form the brush macroinitiator (***BI***).

5. Formation of BASPs

1. Add 3.6±0.1 mg (6.18 µmol, 10 eq to the amount of ***BI*** to be transferred in step 5.2), 5.5 ± 0.1 mg (9.28µmol,15 eq to the amount of ***BI*** to be transferred in step 5.2), and 7.3±0.1 mg (12.4µmol,20 eq to the amount of ***BI*** to be transferred in step 5.2) of crosslinker ***C*** to three separate 3 ml vials equipped with stir bars. Try to weigh the crosslinker directly onto the bottom of the vial to prevent material from adhering to the sides of the vial. Note: Crosslinker ***C*** is not highly soluble in THF. For this reason, the solid is used directly in this step. In cases where the crosslinker is soluble, then a concentrated stock solution of crosslinker can be made and various amounts of this solution can be transferred to vials. Again, the concentration of the final polymerizations should be >0.05 M; if solvent is added to the crosslinker then a compensatory reduction of solvent should be made elsewhere.
2. Add 123 µl (0.618 µmol) of the ***BI*** solution to each of the three vials containing ***C***. Try to keep the needle tip just above the solid crosslinker when adding to the vial. Add the ***BI*** solution all at once rather than dropwise.
3. Cap the vials and stir the reactions at RT for until completion. With this specific MM and crosslinker combination, the reaction is complete in ~4 hr; continued stirring for up to 24 hr has no discernible effect on BASP growth. Monitor by GPC to ensure complete conversion of ***BI***.
4. Quench the reactions by adding one drop of ethyl vinyl ether to the remaining ***BI*** solution and each of the *N* = 10, 15, and 20 BASP reaction mixtures. Stir for 10 min to ensure complete quenching.

6. GPC Sample Preparation

The GPC-MALLS results were obtained on an Agilent 1260 LC system equipped with a Shodex GPC KD-806M column, a Wyatt Dawn Heleos-II MALLS detector, and a Wyatt Optilab t-rEX refractive index detector at room temperature. DMF with 0.025 M LiBr at a flow rate of 1.0 ml/min was used as the eluent. Results were analyzed using Astra 6 software provided by Wyatt.

1. Using a new glass pipette for each reaction vial, dip the pipette tip into the reaction solution to draw up a small sample of the reaction. Wash down the inside of the pipette with 250 µl of 0.025 M LiBr in DMF to give a final concentration of roughly 3 mg/ml.
2. Filter the diluted sample through a 0.45 µm polytetrafluoroethylene filter before depositing the sample into a GPC vial.
3. Set-up GPC-MALLS runs and analyze the results once the runs are completed.

List of Abbreviations:

A: Grubbs 3rd generation bis-pyridine catalyst

B: poly(ethylene glycol) (PEG) macromonomer (MM)

B1: PEG MM prepared using commercially available (Aldrich) PEG-NH_2 and used without HPLC purification.

B2: PEG MM prepared using commercially available (Aldrich) PEG-NH_2 and used after HPLC purification.

B3: PEG MM prepared using newly synthesized PEG-NH_2 and used after HPLC purification.

BASP: brush-arm star polymer

BI: living brush initiator

C: photodegradable crosslinker

Đ: molar mass dispersity index

DMF: *N,N*-dimethylformamide

DP: number average degree polymerization

GPC: gel permeation chromatography

Prep-HPLC: preparative high performance liquid chromatography

MALLS: multi-angle laser light scattering

MM: macromonomer

MW: molecular weight

M_w: weight average molar mass

N: number of crosslinker equivalents (ratio of ***C*** to ***A***)

NHS: *N*-hydroxysuccinimidyl

PEG: polyethylene glycol

PEG-MM: norbornene-PEG macromonomer (also referred to as compound ***B***)

ROMP: ring-opening metathesis polymerization

THF: tetrahydrofuran

Representative Results

Figure 2 shows GPC traces for a variety of BASPs prepared from ***B1***, ***B2***, and ***B3***. In all cases, the data illustrate that increasing the equivalents of crosslinker (*N*) leads to an increase in the size of the BASP. As was observed in our previous report, 10 equivalents of crosslinker is not sufficient to achieve uniform BASPs; the *N* = 10 sample shows a clearly multi-modal GPC trace with a large amount of residual brush polymer especially in the case of unpurified MM ***B1*** (**Figure 2a**). Greater amounts of crosslinker result in uniform MW distributions with very little residual brush and MM. The weight-average molar mass (M_w) approximately doubles in going from *N* = 15- 20. In the case of ***B3***, no residual MM and less than 1% residual BI remains for the *N* = 15 and *N* = 20 cases.

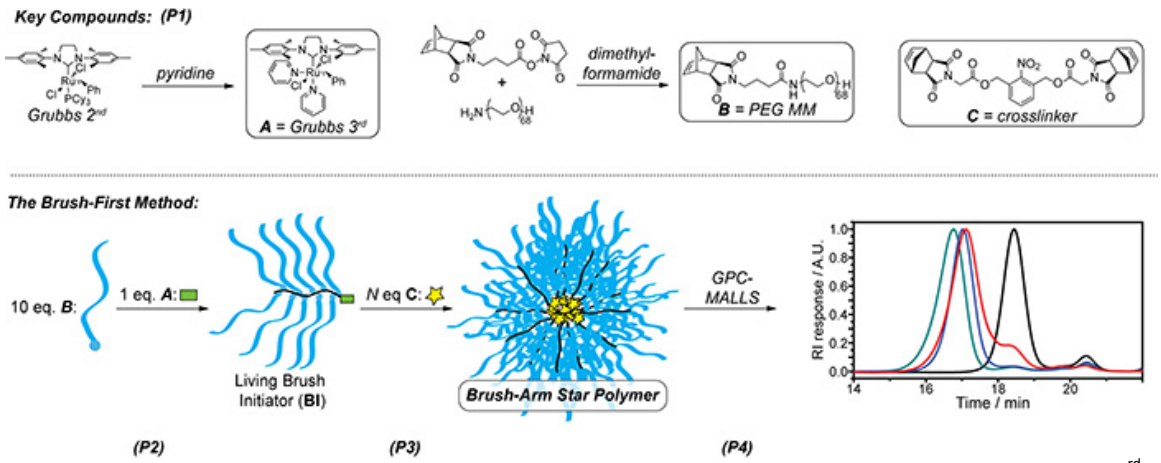

Figure 1. Schematic for Brush-Arm Star Polymer (BASP) Synthesis. Panel (a) illustrates the synthesis of Grubbs' 3rd generation bispyridine catalyst (**A**) from commercially available Grubbs' 2nd-generation catalyst. Also shown are the structures of the PEG-MM (**B**) and crosslinker (**C**) used in this work. Panel (b) shows a schematic diagram of the brush-first process. Polymerization of PEG-MM (**B**) with catalyst (**A**) generates a 10-unit living brush initiator (***BI***), which is then added to crosslinker (**C**) resulting in the formation of a BASP. Click here to view larger image.

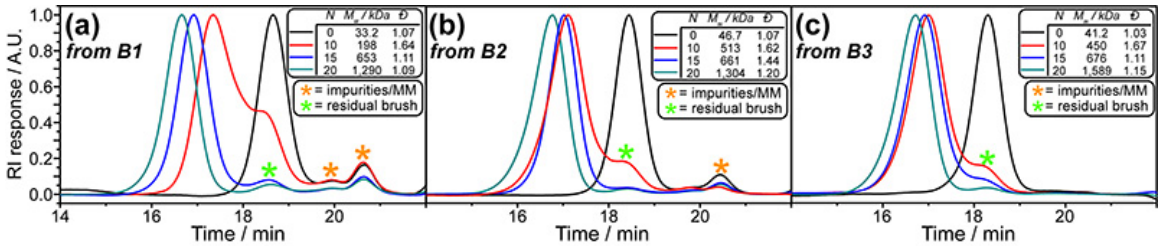

Figure 2. Representative GPC results of the *N* = 10, 15, and 20 BASPs prepared from various PEG MMs. Panels (a), (b), and (c) depict data for MMs **B1**, **B2**, and **B3**, respectively. Impurities from commercial PEG-NH_2, unreacted MM, and residual ***BI*** are labeled with asterisks. M_w and dispersity index (*Đ*) values are provided in the inset tables. Note that *Đ* values obtained by GPC for highly branched nanostructures must be considered carefully[24,25]. The observation of monomodal, uniform peaks suggests a narrow distribution of particle radii. Click here to view larger image.

Discussion

The key advantage of brush-first BASP synthesis is the unique ability to rapidly synthesize nanostructures of diverse size and composition in parallel without need for specialized equipment. In this study, we demonstrate the brush-first synthetic method using a norbornene functionalized PEG macromonomer (***B***, **Figure 1**) and a bis-norbornene nitrobenzyl ester crosslinker (**C**, **Figure 1**). The PEG chains from ***B*** impart water solubility to the final BASP structure. The nitrobenzyl-based crosslinker is photodegradable.

This general procedure can be modified for other *exo*-norbornene based MMs and crosslinkers. We have prepared BASPs from several combinations of both. For example, we have used norbornene-PEG-based MMs that carry different anti-cancer drugs, nitroxides, and magnetic resonance imaging contrast agents [27]. We have also used MMs comprised of polymers other than PEG. In our experience, the brush-first method can be applied to nearly any functional *exo*-norbornene imide terminated MM. In cases where high conversions of MM to ***BI*** (>95%) are not achieved, an MM impurity is the most likely culprit (as opposed to catalytic activity). More rigorous purification as outlined in this report (prep-HPLC) typically leads to successful ROMP. Note that we have not attempted ROMP polymerizations with MMs that bear unprotected functional groups that are known to interfere with catalyst **A** (*e.g.* free amines, olefins, azides, *etc.*). These groups can be introduced after the brush-first synthesis via post-polymerization modification[27]. For example, we have prepared azide-BASPs from alkyl halide MMs that were converted to azides after BASP formation. These azides were used for Cu-catalyzed azide-alkyne cycloaddition "click" reactions.

We sought to study the impact of MM purity in more detail. Small amounts of residual MM and ***BI*** were always observed in GPC traces when brush-first reactions were carried out using MM prepared from commercially available PEG-NH_2 (**B1**, **Figure 2a**). We had learned from experience that completely pure MMs generally give quantitative MM conversion. Furthermore, we had noticed that the amount of residual MM varied depending on the batch number of the commercial PEG-NH_2. We suspected that a non-functional PEG-NH_2 impurity, perhaps simply PEG diol, was responsible for the apparent residual MM impurity. Therefore, we utilized prep-HPLC to purify **B1** to give pure MM **B2**. **Figure 2b** shows that this purification process did indeed decrease the amount of residual MM (orange star) approximately two-fold; it did not remove it completely. Interestingly, **B2** gave higher conversion of ***BI*** to BASPs as well; perhaps an impurity that led to catalyst deactivation was removed via prep-HPLC. Still dissatisfied with the amount of residual MM, we followed literature methods for the synthesis of PEG-NH_2 via anionic polymerization of ethylene oxide from ethanolamine (CAUTION: Ethylene oxide should be handled by trained, experienced chemists; it is a highly flammable, explosive, and toxic gas!).[22,23] MM prepared from this homemade PEG-NH_2 (**B3**) yielded improved results compared to the commercial MMs. GPC analysis of the corresponding BASPs showed no detectable residual MM and very little (<1%) residual ***BI*** (**Figure 2c**). Thus, if high purity BASPs are required we recommend using the purest possible MM. Note that residual MM and ***BI*** can readily be removed from the larger BASPs through dialysis after brush-first synthesis.

We have also used crosslinkers other than **C**. For example, we have prepared BASPs from bisnorbornene metal complexes, polymerization initiators, acid-cleavable linkers, and supramolecular hosts. We find that crosslinkers with rigid spacers between the norbornenes tend to provide the most uniform BASPs; such crosslinkers are less likely to undergo intramolecular cyclization reactions that consume norbornenes but do not contribute to BASP growth.

Regardless of the MM and crosslinker combination, we find the following general practices will lead to the highest chance of brush-first success. First, before attempting brush-first synthesis with newly synthesized monomers, we recommend making the DP = 10 brush polymer alone and possibly longer brush polymers with DP = 25 and 50. If these tests are successful, there is an excellent chance that the brush-first method will also be successful. Second, the ideal concentration for the brush-first polymerization is dependent on monomer chemical composition and structure of the components. We recommend testing a few concentrations on small scale before making a large batch of BASP. Third, polymerizations carried out in dichloromethane or tetrahydrofuran appear to give the best results; monomers that are soluble in these solvents are ideal. As discussed above, if the crosslinker is poorly soluble in these solvents we recommend adding it as a solid rather than adding extra solvent. As long as the MM is soluble, we find that the crosslinking brings the crosslinker completely into solution within minutes. Fourth, though the polymerization does not require inert conditions, we recommend storage of the catalyst under inert atmosphere to increase its lifetime. Importantly, the catalyst will decompose over time in solution; the catalyst solution should be prepared fresh from the Grubbs third generation catalyst each time a series of ROMP reactions is performed. Finally, the amount of crosslinker required for uniform BASPs will vary widely with crosslinker and MM structure. As shown in **Figure 2**, 10 equiv of crosslinker **C** is not enough to provide complete ***BI*** conversions. In other cases, we find that addition of 1 equiv of crosslinker, and even up to 40 equiv, provides good results. Whenever a new crosslinker is to be used, we recommend running a series of small-scale reactions with various *N* values to identify optimal crosslinker amounts.

As a final note, it is important recognize that many alternative methods exist for making star shaped polymers (core-first, arm-first, *etc.*)[25, 26]. Each method has disadvantages and advantages, such as limits to size, purification requirements, and functional group compatibility. We argue that the broad functional group tolerance of ROMP, the ease of synthesis of norbornene-based functional monomers, and the ability to perform ROMP reactions on the benchtop rapidly, in parallel, and at room temperature, make the brush-first ROMP approach worth consideration for a variety of applications. In the future, we will continue to develop this method and BASP nanoarchitectures for various applications including drug and gene delivery, cellular imaging, and self-assembly. The full potential of these novel particles, and their capacity for combinatorial synthesis, has yet to be explored.

Disclosures

The authors have nothing to disclose.

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References

1. Bielawski, C. W. Grubbs, R. H. Living ring-opening metathesis polymerization. *Prog. Polym. Sci.* **32**, 1-29 (2007).
2. Hawker, C. J.The Convergence of Synthetic Organic and Polymer Chemistries. *Science.* **309**, 1200-1205, (2005).
3. Peer, D. Karp, J. M. Hong, S. Farokhzad, O. C. Margalit, R. Langer, R. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nano.* **2**, 751-760 (2007).
4. Whitesides, G. M. Nanoscience, Nanotechnology, and Chemistry. *Small*. **1,** 172-179 (2005).
5. Leitgeb, A. Wappel, J. Slugovc, C. The ROMP toolbox upgraded. *Polymer.* **51**, 2927-2946 (2010).
6. Johnson, J. A. Lu, Y. Y. Burts, A. O. Lim, Y.-H. Finn, M. G. Koberstein, J. T. Turro, N. J. Tirrell, D. A. Grubbs, R. H. Core-Clickable PEG-Branch-Azide Bivalent-Bottle-Brush Polymers by ROMP: Grafting-Through and Clicking-To. *J. Am. Chem. Soc.* **133**, 559-566 (2010).
7. Bielawski, C. W. Grubbs, R. H. Highly Efficient Ring-Opening Metathesis Polymerization (ROMP) Using New Ruthenium Catalysts Containing N-Heterocyclic Carbene Ligands. *Angew. Chem. Int. Ed.* **39**, 2903-2906 (2000).
8. Love, J. A. Morgan, J. P. Trnka, T. M. Grubbs, R. H. A Practical and Highly Active Ruthenium-Based Catalyst that Effects the Cross Metathesis of Acrylonitrile. *Angew. Chem. Int. Ed.* **41**, 4035-4037 (2002).
9. Johnson, J. A. Lu, Y. Y. Burts, A. O. Xia, Y. Durrell, A. C. Tirrell, D. A. Grubbs, R. H. Drug-Loaded, Bivalent-Bottle-Brush Polymers by Graft-through ROMP. *Macromolecules.* **43**, 10326-10335 (2010).
10. Burts, A. O. Li, Y. J. Zhukhovitskiy, A. V. Patel, P. R. Grubbs, R. H. Ottaviani, M. F. Turro, N. J. Johnson, J. A. Using EPR To Compare PEG-branch-nitroxide "Bivalent-Brush Polymers" and Traditional PEG Bottle-Brush Polymers: Branching Makes a Difference. *Macromolecules.* **45**, 8310-8318 (2012).
11. Bazan, G. C. Schrock, R. R. Synthesis of star block copolymers by controlled ring-opening metathesis polymerization. *Macromolecules.* **24**, 817-823 (1991). .
12. Saunders, R. S. Cohen, R. E. Wong, S. J. Schrock, R. R. Synthesis of amphiphilic star block copolymers using ring-opening metathesis polymerization. *Macromolecules.* **25**, 2055-2057 (1992).
13. Buchmeiser, M. R. Wurst, K. Access to Well-Defined Heterogeneous Catalytic Systems via Ring-Opening Metathesis Polymerization (ROMP): Applications in Palladium(II)-Mediated Coupling Reactions. *J. Am. Chem. Soc.* **121**, 11101-11107 (1999).
14. Weichelt, F. Frerich, B. Lenz, S. Tiede, S. Buchmeiser, M. R. Ring-Opening Metathesis Polymerization-Based Synthesis of $CaCO_3$ Nanoparticle-Reinforced Polymeric Monoliths for Tissue Engineering. *Macromol. Rapid Comm.* 31, 1540-1545 (2010).
15. Weichelt, F. Lenz, S. Tiede, S. Reinhardt, I. Frerich, B. Buchmeiser, M. R. ROMP-Derived cyclooctene-based monolithic polymeric materials reinforced with inorganic nanoparticles for applications in tissue engineering. *Beilstein J. Org. Chem.* **6**, 1199-1205 (2010).
16. Mayr, M. Mayr, B. Buchmeiser, M. R. Monolithic Materials: New High-Performance Supports for Permanently Immobilized Metathesis Catalysts. *Angew. Chem. Int. Ed.* **40**, 3839-3842 (2001).

17. Mayr, B. Hölzl, G. Eder, K. Buchmeiser, M. R. Huber, C. G. Hydrophobic, Pellicular, Monolithic Capillary Columns Based on Cross-Linked Polynorbornene for Biopolymer Separations. *Anal. Chem.* **74**, 6080-6087 (2002).
18. Otani, H. Fujita, S. Watanabe, Y. Fujiki, M. Nomura, K. A Facile, Controlled Synthesis of Soluble Star Polymers Containing a Sugar Residue by Ring-Opening Metathesis Polymerization (ROMP). *Macromol. Symp.* **293**, 53-57 (2010).
19. Nomura, K. Watanabe, Y. Fujita, S. Fujiki, M. Otani, H. Facile Controlled Synthesis of Soluble Star Shape Polymers by Ring-Opening Metathesis Polymerization (ROMP). *Macromolecules.* **42**, 899-901 (2009).
20. Gao, H. Ohno, S. Matyjaszewski, K. Low Polydispersity Star Polymers via Cross-Linking Macromonomers by ATRP. *J. Am. Chem. Soc.* **128**, 15111-15113 (2006).
21. Liu, J. Burts, A. O. Li, Y. Zhukhovitskiy, A. V. Ottaviani, M. F. Turro, N. J. Johnson, J. A. "Brush-First" Method for the Parallel Synthesis of Photocleavable, Nitroxide-Labeled Poly(ethylene glycol) Star Polymers. *J. Am. Chem. Soc.* **134**, 16337-16344 (2012).
22. Studer, P. Larras, V. Riess, G. Amino end-functionalized poly(ethylene oxide)-block-poly(methylidene malonate 2.1.2) block copolymers: synthesis, characterization, and chemical modification for targeting purposes. *Eur. Polym. J.* **44**, 1714-1721 (2008).
23. Mosquet, M. Chevalier, Y. Le Perchec, P. Guicquero, J. P. Synthesis of poly (ethylene oxide) with a terminal amino group by anionic polymerization of ethylene oxide initiated by aminoalcoholates. *Macromol. Chem. Phys.* **198**, 2457-2474 (1997).
24. Burchard, W. Solution properties of branched macromolecules. *Adv. Polym. Sci.* **143**, 113-194 (1999).
25. Gao, H. F. Development of Star Polymers as Unimolecular Containers for Nanomaterials. *Macromol. Rapid Comm.* **33**, 722-734 (2012).
26. Blencowe, A. Tan, J. F. Goh, T. K. Qiao, G. G. Core cross-linked star polymers via controlled radical polymerisation. *Polymer.* **50**, 5-32 (2009).
27. Burts, A. O. Liao, L. Lu, Y. Y. Tirrell, D. A. Johnson, J. A. Brush-first and Click: Efficient Synthesis of Nanoparticles that Degrade and Release Doxorubicin in Response to Light. *Photochem. Photobiol.* DOI: 10.1111/php.12182 (2013).

