JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
pubs.acs.org/JACS

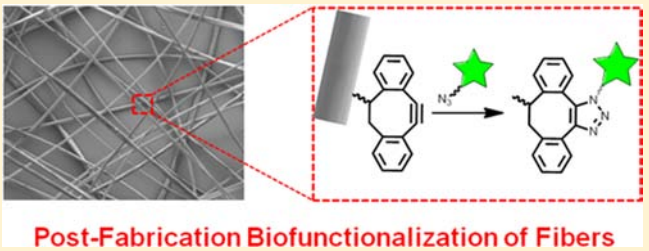
Post-Assembly Derivatization of Electrospun Nanofibers via Strain-Promoted Azide Alkyne Cycloaddition

Jukuan Zheng,[†] Kaiyi Liu,[†] Darrell H. Reneker,[†] and Matthew L. Becker*[,†,‡]

[†]Department of Polymer Science, The University of Akron, Akron, Ohio 44325, United States
[‡]Center for Biomaterials in Medicine, Austen Bioinnovation Institute in Akron, Akron, Ohio 44308, United States

Supporting Information

ABSTRACT: A primary amine-derivatized 4-dibenzocyclooctynol (DIBO) was used to initiate the ring-opening polymerization of poly(γ-benzyl-L-glutamate) (DIBO-PBLG). This initiator yields well-defined PBLG polymers functionalized with DIBO at the chain termini. The DIBO end group further survives an electrospinning process that yields nanofibers that were then derivatized post-assembly with azide-functionalized gold nanoparticles. The availability of DIBO on the surface of the fibers is substantiated by fluorescence, SEM, and TEM measurements. Post-assembly functionalization of nanofiber constructs with bioactive groups can be facilitated easily using this process.



Polymeric nanofibers are applied increasingly to regenerative medicine applications due to dimensional similarities with collagen fibers in the natural extracellular matrix.[1] Nanofibers are fabricated using melt- or electrospinning processes that are able to control both size and morphology using a variety of conditions including solvent, concentration, and additives.[2] Many of the reported fiber technologies have focused on using polymeric materials, which are utilized in FDA-approved applications or devices. While useful, these materials in general lack specific functionality that would guide or direct specific biological functions.[3] For regenerative medicine, fabrication of biomolecule-derivatized nanofibers that enhance cell−material interactions is important.[4] The derivitization of nanofibers often requires multiple procedures, including plasma treatment, wet chemical methods, surface graft polymerization, and co-electrospinning of surface active agents with polymers.[3] Each of these modifications are time- and resource-intensive to optimize and may lead to immune specific reactions and biocompatibility problems.[5] Recently, click chemistry,[6,7] especially strain-promoted azide-alkyne cycloaddition (SPAAC) reactions, has emerged as a promising biofunctionalization method due to its high efficiency, orthogonality, and tolerance of reaction conditions in the presence of cellular systems.[8] SPAAC has been investigated in biological hydrogels,[9−11] polymer side-chain functionalization,[12] and as a labeling method for biological systems,[13,14] among others. Several generations of cyclooctynes have been developed recently by both the Boons and Bertozzi groups.[15,16]

To our knowledge, amine-derivatized cyclooctynes have not been utilized previously as an initiator for ring-opening polymerization. In this work, 4-dibenzocyclooctynol (DIBO) functionalized with a primary amine group was used as an initiator for the ring-opening polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride (Bz-L-GluNCA) to yield a 4-dibenzocyclooctynol functionalized poly(γ-benzyl-L-glutamate) (DIBO-PBLG). PBLG is a versatile, degradable material that can adopt α-helix and β-sheet conformations[17] and is being investigated for cell adhesion and proliferation when used with protein preadsorption techniques.[18,19] The high binding affinity of calcium to PBLG is also promising for bone regeneration applications.[20]

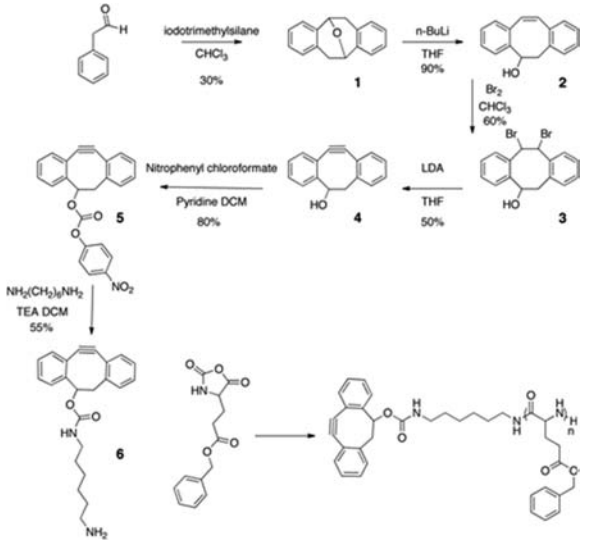
The DIBO-PBLG was synthesized as described in Scheme 1. The strained DIBO precursor was synthesized according to previously described methods.[15,21] The DIBO was derivatized with *p*-nitrophenyl chloroformate and further reacted with excess hexamethylene diamine to yield the primary amine-derivatized DIBO compound, **6**. Bz-L-GluNCA was synthesized and purified by flash chromatography according to previously reported procedures.[22,23]

The ^{1}H NMR spectrum of the DIBO initiator and DIBO-PBLG are shown in Figure 1. The initiator was used immediately after purification. In a series of polymerizations the amine-derivatized DIBO was used as an initiator for the ring-opening polymerization of Bz-L-GluNCA in anhydrous DMF under nitrogen for 3 days to yield DIBO-functionalized PBLG. The molecular weight of DIBO-PBLG increased linearly with increasing feed ratio from 50:1 to 500:1. The corresponding molecular weight distribution increased from 1.14 to 1.29, which demonstrated that these polymerizations are well controlled and exhibit linear growth kinetics with increasing feed ratio. The various feed ratio conditions and the resulting molecular weights and molecular weight distributions of the resulting polymers were measured via DMF phase SEC

Received: August 2, 2012
Published: September 26, 2012

dx.doi.org/10.1021/ja307647x | J. Am. Chem. Soc. 2012, 134, 17274−17277

Scheme 1. Synthesis of Dibenzocyclooctynol Functionalized Poly(γ-benzyl-L-glutamate) (DIBO-PBLG)



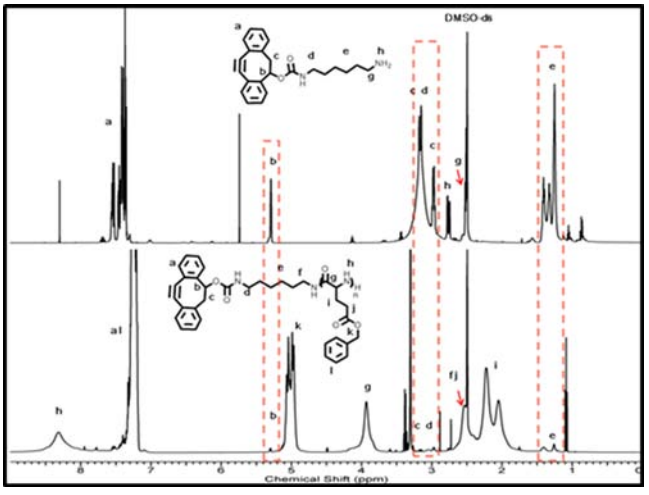


Figure 1. ^{1}H NMR spectrum of the DIBO-derivatized initiator and DIBO-functionalized PBLG in DMSO-d_6.

and are shown in Figure 2. The high molecular mass shoulders that appear at increasing feed ratios are consistent with what has been reported by others using amine -based initiators for the polymerization of PBLG and are most likely due to water-initiated polymer.[24,25]

The DIBO-derivatized PBLG was then used as a functional precursor for electrospinning of nanofibers to generate a copper-free clickable scaffold. DIBO-PBLG (M_n = 128 K, PDI = 1.29) and the unmodified PBLG (M_w 150,000−350,000, Sigma) were both prepared in a 12 wt % 1,4-dioxane solution. Each polymer solution was held in a glass pipet and was electrospun from the orifice having an inner diameter of 300 μm at the tip. The electric potential was 6 kV over a 22 cm tip-to-collector distance for the modified polymer and 12 kV over a 27 cm tip-to-collector distance for the unmodified polymer. A proper positive air pressure was applied on the surface of the solution to maintain the feeding rate. Fibers were collected on conductive glass slides for the subsequent fluorescence measurements, silicon wafers for SEM observation, and on copper grids for TEM observation. Each type of collector was placed on top of a large grounded aluminum foil mat for the

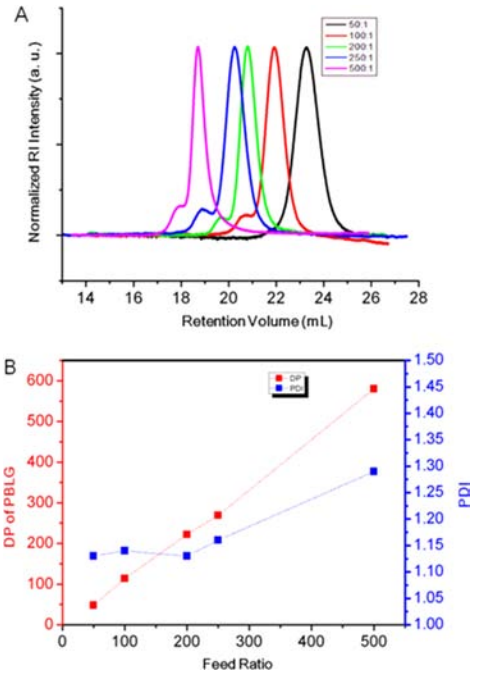


Figure 2. SEC results of DIBO-PBLG with different feed ratios. SEC was run in DMF with LiBr (0.1 mol/L) under 50 °C.

collection of electrospun fibers. Samples were silver coated with an SPI sputter coater before the SEM observation. From SEM micrographs, we obtained fibers with diameters near 1 μm in diameter (Figure 3).

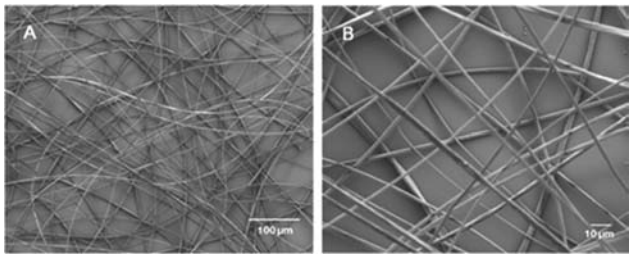


Figure 3. SEM pictures of nanofibers from electrospinning show highly uniform fiber diameters.

To confirm the survival of the strained DIBO group following the electrospinning process, the fibers were dissolved in DMF, and UV−vis spectra of the solutions showed the presence of optical transitions near 306 nm, which correspond to the alkyne group in DIBO (Figure 4), before and after the fabrication process. With the survival of DIBO group through electrospinning, the availability of DIBO on the surface of fibers for biofunctionalization was probed.

The extent of DIBO available on the surface of the nanofibers to react was quantified using UV−vis spectroscopy. A solution of 9-methyleneazidoanthracene in methanol, which is a non-solvent for PBLG, was allowed to react with a mat of electrospun fibers. Concurrently, a solution of 9-methyleneazidoanthracene in *N*,*N*-dimethyl formamide, which is a good solvent for PBLG, was allowed to react with an equivalent solution of the DIBO-initiated PBLG. The absorbance of the nanofiber post-functionalization and PBLG-DIBO with identical concentrations was measured following the reaction, and from the reduction in absorbance of the alkyne transition in

dx.doi.org/10.1021/ja307647x | J. Am. Chem. Soc. 2012, 134, 17274−17277

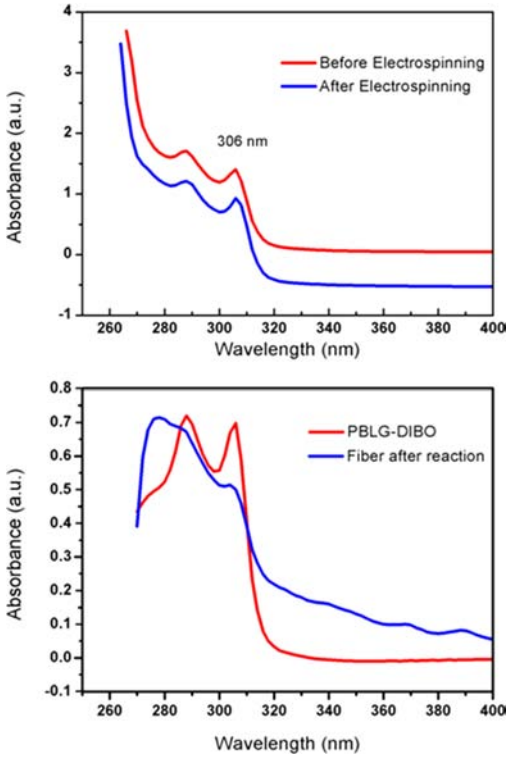


Figure 4. UV spectra of DIBO-PBLG before and after electrospinning indicate that the strained cyclooctyne survives the scaffold fabrication process (top). A second experiment measuring the residual DIBO left following a cycloaddition reaction, which indicated approximately 26% of the groups were able to react (bottom).

DIBO, the fraction of the DIBO group on the surface of fiber that is available for derivatization is 26 ± 5%.

A post-assembly experiment utilizing an azide-containing fluorescence probe (Chremo 488 azide) was conducted. A glass coverslip containing fibers was immersed in a 0.4% (mg/mL) solution of fluorescence probe for 5 min. Following the brief incubation, the fiber-loaded coverslip was removed, washed with methanol, and dried under nitrogen. Using fluorescence microscopy, we clearly showed that the azide-functionalized fluorescence probe reacted with the DIBO group on the surface of fibers. Underivatized PBLG exhibited no fluorescence following the methanol wash (Figure 5). These experiments indicate the availability of the DIBO groups on the surface for functionalization through strain-promoted azide-alkyne cycloaddition.

Further proof of the existence of DIBO group on the surface of the fibers was obtained from SEM micrographs of the fibers following immersion in a solution of azide-functionalized gold nanoparticles. Fiber-loaded silicon wafers were immersed in a solution of azide-functionalized gold nanoparticles (50 nm, Nanocs, diluted 500 times) for 5 h, after which the wafer was washed with nanopure water (18 MΩ/cm^{-1}) and dried under vacuum. The SEM images clearly showed the presence of gold nanoparticles on the surface of the fibers, which confirmed that the DIBO group on the surface of fibers are available for functionalization (Figure 6 A,B). A control experiment was performed using fibers composed of unmodified PBLG, which showed conclusively that the nonspecific physical adsorption of gold nanoparticles to the surface of fibers is negligible (Figure 6 C,D).

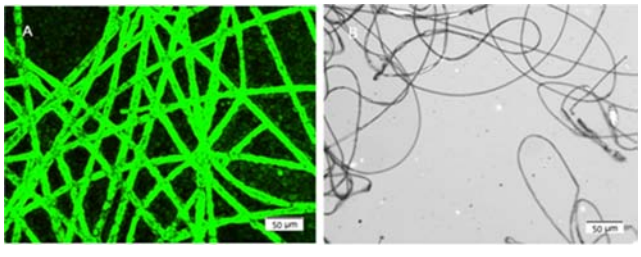


Figure 5. Fluorescence images of ChremoTM azide stained fibers (A) and an optical image of unmodified fibers (B).

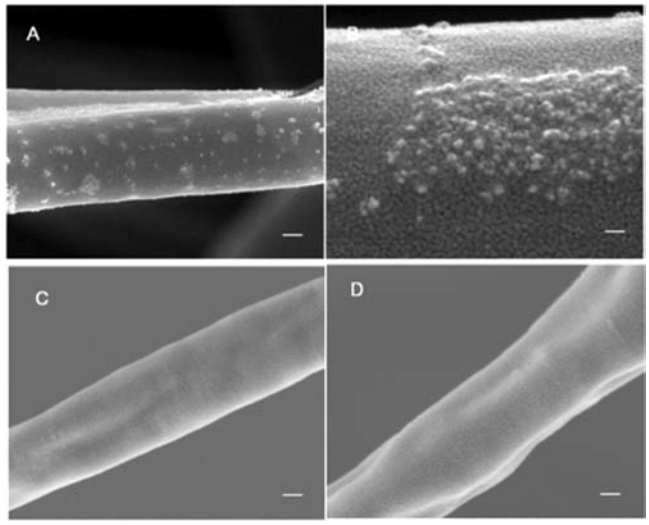


Figure 6. SEM micrographs of gold nanoparticle (50 nm) functionalized fibers (A, B) and unmodified fibers as control (C, D). The scale bar for images A, B, C, and D is 600, 100, 300, and 200 nm, respectively.

Additional experiments with transmission electron microscopy (TEM) confirmed the existence and availability of DIBO groups on the surface of the fibers. TEM grids loaded with nanofibers were immersed in an azide-functionalized gold nanoparticle solution for 1 h at ambient temperature, after which the grid was washed with nanopure water (18 MΩ/cm^{-1}) and dried under vacuum. TEM images showed that gold nanoparticles are present on the surface of the nanofibers. Control experiments with unmodified nanofibers showed that no nonspecific physical adsorption of gold nanoparticles was evident following immersion in the nanoparticle containing solution (Figure 7). Due to the relatively large size of nanofibers (chosen for visualization purposes), only small portions of the fibers are shown in the SEM and TEM micrographs.

In conclusion, we demonstrate the utility of an amine-derivatized DIBO unit as an initiator for ring-opening polymerization for the first time. Additionally we show that the DIBO group survives an electrospinning procedure. The resulting nanofiber scaffold is then available for post-assembly functionalization with any number of azide-derivatized molecules. The availability of copper-free click-chemistry-based biofunctionalization sites on the surface of nanofibers offers versatile approach to create highly functional scaffolds, which we feel will be useful in a variety of promising applications in regenerative medicine.

dx.doi.org/10.1021/ja307647x | J. Am. Chem. Soc. 2012, 134, 17274−17277

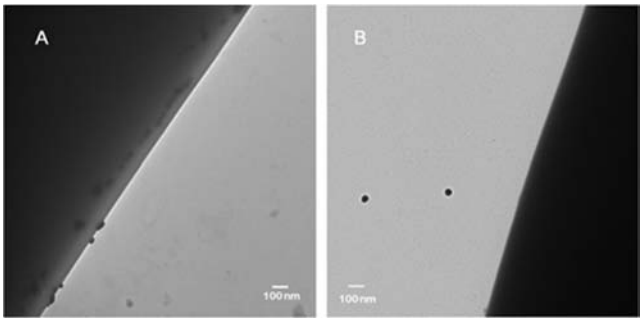


Figure 7. TEM images of gold nanoparticle (50 nm) functionalized fibers (A) and unmodified fibers (B).

■ ASSOCIATED CONTENT

Supporting Information

Synthetic details and characterization of the DIBO and amine-derivatized DIBO. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

becker@akron.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from the Akron Functional Materials Center and the Austen Bioinnovation Institute in Akron.

■ REFERENCES

(1) Ma, Z.; Kotaki, M.; Inai, R.; Ramakrishna, S. *Tissue Eng.* **2005**, *11*, 101−109.
(2) Reneker, D. H.; Yarin, A. L. *Polymer* **2008**, *49*, 2387−2425.
(3) Yoo, H. S.; Kim, T. G.; Park, T. G. *Adv. Drug Delivery Rev.* **2009**, *61*, 1033−1042.
(4) Pham, Q. P.; Sharma, U.; Mikos, A. G. *Tissue Eng.* **2006**, *12*, 1197−1211.
(5) Kim, B. J.; Choi, Y. S.; Cha, H. J. *Angew. Chem., Int. Ed* **2012**, *51*, 675−678.
(6) Barner-Kowollik, C.; Du Prez, F. E.; Espeel, P.; Hawker, C. J.; Junkers, T.; Schlaad, H.; Van Camp, W. *Angew. Chem., Int. Ed.* **2011**, *50*, 60−62.
(7) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021.
(8) Lutz, J. F. *Angew. Chem., Int. Ed.* **2008**, *47*, 2182−2184.
(9) DeForest, C. A.; Polizzotti, B. D.; Anseth, K. S. *Nat. Mater.* **2009**, *8*, 659−664.
(10) DeForest, C. A.; Anseth, K. S. *Nat. Chem.* **2011**, *3*, 925−931.
(11) Zheng, J.; Smith-Callahan, L. A.; Hao, J.; Guo, K.; Wesdemiotis, C.; Weiss, R. A.; Becker, M. L. *ACS Macro Lett.* **2012**, *1*, 1071−1073.
(12) Zeng, D.; Lee, N. S.; Liu, Y.; Zhou, D.; Dence, C. S.; Wooley, K. L.; Katzenellenbogen, J. A.; Welch, M. J. *ACS Nano* **2012**, *6*, 5209−5219.
(13) Poloukhtine, A. A.; Mbua, N. E.; Wolfert, M. A.; Boons, G.-J.; Popik, V. V. *J. Am. Chem. Soc.* **2009**, *131*, 15769−15776.
(14) Yao, J. Z.; Uttamapinant, C.; Poloukhtine, A.; Baskin, J. M.; Codelli, J. A.; Sletten, E. M.; Bertozzi, C. R.; Popik, V. V.; Ting, A. Y. *J. Am. Chem. Soc.* **2012**, *134*, 3720−3728.
(15) Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G.-J. *Angew. Chem., Int. Ed.* **2008**, *47*, 2253−2255.
(16) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974−6998.
(17) Papadopoulos, P.; Floudas, G. *Biomacromolecules* **2004**, *5*, 81−91.
(18) Webster, T. J.; Ergun, C.; Doremus, R. H.; Siegel, R. W.; Bizios, R. *J. Biomed. Mater. Res.* **2000**, *51*, 475−483.
(19) Webster, T. J.; Siegel, R. W.; Bizios, R. *Biomaterials* **1999**, *20*, 1221−1227.
(20) Ravichandran, R.; Venugopal, J. R.; Sundarrajan, S.; Mukherjee, S.; Ramakrishna, S. *Biomaterials* **2012**, *33*, 846−855.
(21) Mbua, N. E.; Guo, J.; Wolfert, M. A.; Steet, R.; Boons, G. J. *ChemBioChem* **2011**, *12*, 1912−1921.
(22) Kramer, J. R.; Deming, T. J. *Biomacromolecules* **2010**, *11*, 3668−3672.
(23) Poché, D. S.; Moore, M. J.; Bowles, J. L. *Synth. Commun.* **1999**, *29*, 843−854.
(24) Lu, H.; Cheng, J. *J. Am. Chem. Soc.* **2007**, *129*, 14114−14115.
(25) Tang, H.; Zhang, D. *Biomacromolecules* **2012**, *11*, 1585−1592.