Controlled Microstructuring of Janus Particles Based on a Multifunctional Poly(ethylene glycol)

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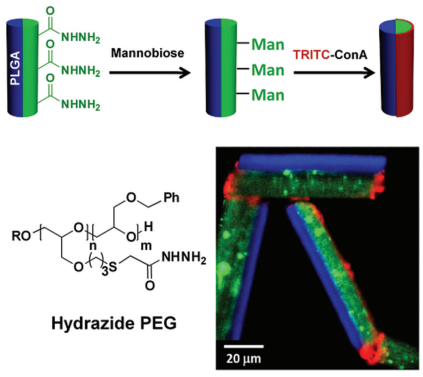
A novel water insoluble, multifunctional poly(ethylene glycol), poly(hydrazide ethylene glycol-*co*-benzyl glycidyl ether) (P(HZ-*co*-BnGE)), is synthesized via thiol-ene click reaction of poly(allyl glycidyl ether-*co*-benzyl glycidyl ether) (P(AGE-*co*-BnGE)). The base polymer P(AGE-*co*-BnGE) is previously prepared by anionic ring-opening copolymerization of the corresponding monomers. To demonstrate utility, bicompartmental microspheres and microcylinders containing P(HZ-*co*-BnGE) in one of the compartments are prepared via electrohydrodynamic (EHD) co-jetting. Next, spatially controlled surface reactivity toward sugars is demonstrated by selective binding of 2α-mannobiose to the P(HZ-*co*-BnGE) compartment only, as confirmed by a carbohydrate-lectin-binding assay. These sugar-reactive hydrazide-presenting microparticles have potential applications for glyco-targeted drug delivery.



1. Introduction

The demand for nano- and microparticles in biomedicine,[1] electronics,[2] optics,[3] and catalysis[4] has grown dramatically in recent years due to the discovery of specific properties and functions imparted by their size. In biomedicine, great progress has been achieved with the use of nano- and microparticles for targeted drug and gene delivery,[5] tissue engineering,[6] and medical diagnostics.[7] Chemical composition as well as physical characteristics, such as size, shape, and mechanical properties of particles are important factors, as they greatly influence particle interactions with tissue, circulation time in blood, cellular uptake, or the ability to release drugs.[5,8] In addition, fabrication of anisotropic particles offers interesting perspectives for advanced drug delivery allowing for combined delivery and imaging,[9] or the delivery of two distinct drugs with different release kinetics.[10]

Various techniques are known to create anisotropic particles with specific properties,[11] including matrix embedment,[12] pickering emulsion polymerization,[13] glancing angle deposition,[14] microfluidics,[15] and electrohydrodynamic (EHD) co-jetting.[16] Major progress was made, when surface anisotropy was introduced generating so-called patchy particles.[12] In comparison, methods for creating particles with bulk anisotropy are still more limited and often involve microfluidic processing[15] or EHD

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co-jetting.[16] The latter is a highly reproducible process, which allows for variation of several particle parameters, including size and shape, and introduction of bulk anisotropy resulting in multicompartmental particles.[16]

In a typical setup of EHD co-jetting, two jetting solutions are transferred through a side-by-side capillary system under a laminar flow regimen. The applied electrical field causes distortion of a droplet at the tip of the nozzle into a so-called Taylor cone and the formation of a very thin polymer thread. Ultrafast solvent evaporation results in precipitation and solidification of the particles before they reach the substrate surface. Particles or fibers can thus be fabricated depending on jetting conditions such as polymer concentration in the jetting solutions and flow rate.[17]

Various polymers have been used for EHD co-jetting. The specific choice of the polymer is usually dictated by the intended application, while general criteria for the polymer selection aimed for biomedical applications remain biocompatibility and biodegradability. Poly(lactide-*co*-glycolide) (PLGA) is most commonly used for generation of particles by EHD co-jetting, as it satisfies the aforementioned criteria and is commercially available.[17] A range of different polymers, such as poly(ethylene glycol) (PEG), polyacrylic acid (PAA), and polyethylene imine (PEI), have already been used for co-jetting in water solutions;[18] however, EHD co-jetting in water solutions requires further cross-linking of the generated particles in order to avoid their dissolution under physiological conditions. This can be achieved with the use of chemical cross-linkers for photochemical or thermal cross-linking reactions. However, these reactions may alter the properties of potential drugs to be loaded in the microparticles. Thus, the jetting of water-insoluble polymers in organic solutions may be preferred.

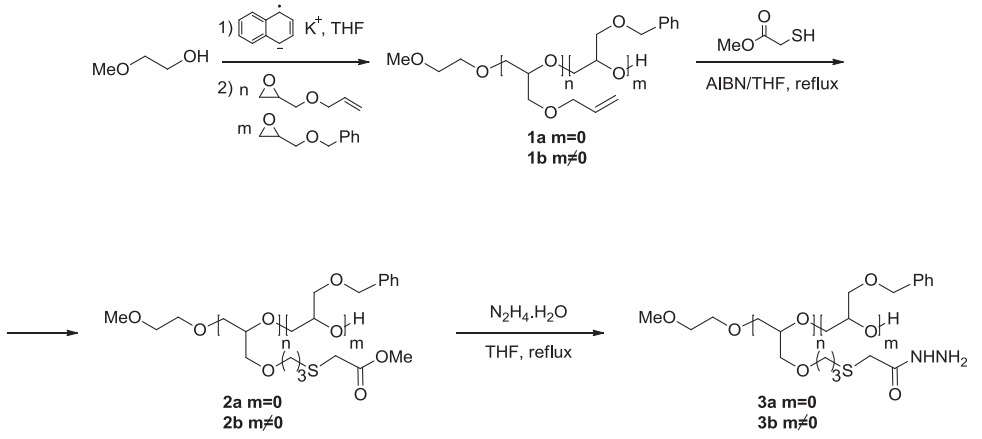
Hence, a growing interest in new functional PEG derivatives has arisen. The PEG polymer has already found diverse applications in biomedicine[19] due to its unique biocompatibility, high protein resistance, and low toxicity and immunogenicity.[20] Attempts to overcome PEG's main drawback—the presence of just two hydroxyl groups at the termini—resulted in the synthesis of various α,ω-telechelic PEGs[21] as well as PEGs with different architectures such as dendrimer- or star-like PEGs.[22] However, the greatest success in introducing maximum functional groups per polymer molecule was achieved by the polymerization of functional epoxides, resulting in linear multifunctional PEGs.[23] To date, chemical groups introduced directly during anionic polymerization of substituted epoxides include alkene, protected hydroxyl, and amino groups.[23] A variety of multifunctional PEGs were also synthesized by postpolymerization modification of polyglycidol[24] and poly(allyl glycidyl ether) (PAGE).[25] However, very few examples exist for functional PEG polymers with high reactivity and selectivity toward targeting ligands, such as sugars or glycoproteins.

Among others, PEG derivatives with a high concentration of hydrazide groups are particularly attractive as hydrazide groups are known to react specifically with aldehydes at ambient conditions, forming effective, yet potentially reversible hydrazone linkages. These groups have particular advantages in particles designed for the delivery of aldehyde-containing drugs[26] or for glycotargeting.[27] A block copolymer of methoxy poly(ethylene glycol) (mPEG) and multifunctional hydrazide PEG (mPEG-*b*-PHZ) has been synthesized by Hruby et al.[26] and used for doxorubicin conjugation and fabrication of micelles. Similarly, Zhou et al.[28] modified this delivery system by synthesizing an analogous random copolymer P(EG-*co*-HZ), which after immobilization of doxorubicin resulted in a water-soluble conjugate. However, these reported polymers are unfortunately not suitable for EHD co-jetting because of their hydrophilicity and incompatibility with organic jetting solvents, such as chloroform.

Here, we report on the synthesis of a new water-insoluble multifunctional hydrazide PEG by sequential thiol-ene modification of custom-made P(AGE-*co*-BnGE), which is synthesized directly via anionic ring-opening copolymerization. Once synthesized, the corresponding ester PEG was further modified by reaction with hydrazine. The resultant polymer was then used for the preparation of bicompartmental microparticles and microcylinders via EHD co-jetting in organic solvents. Moreover, the reactivity of the newly synthesized bicompartmental particles toward sugars was confirmed by spatially selective surface modification via carbohydrate-lectin binding.

2. Results and Discussion

The synthesis of linear PHZ (**3a**) was achieved by a two-step modification of PAGE (**1a**) (Scheme 1), similar, to synthesis of diblock copolymer mPEG-*b*-PHZ.[26] To adapt water-soluble homo-multifunctional PHZ for EHD co-jetting, its hydrophilic properties need to be adjusted. Increasing the hydrophobicity of the polymer can be achieved via increasing the ratio of apolar to polar comonomeric units. Here, we pursued the introduction of hydrophobic moieties (phenyl groups) into the polymer structure to enhance the hydrophobicity of the PEG derivative. This was achieved via copolymerization of AGE with epoxide-bearing hydrophobic phenyl group. Subsequent introduction of the hydrazide groups was performed via sequential thiol-ene click modification and reaction with hydrazine. The polymerization of different glycidyl ethers including AGE has been previously investigated.[23,24b,29] In our study, we found potassium naphthalenide to be most suitable for the formation of the initiating alkoxide moieties. The



Scheme 1. Synthesis of hydrazide-modified PEG derivatives.

conversion of an alcohol into an alkoxide proceeds quantitatively and is easily controlled by the obvious color change. Furthermore, polymerization proceeds under mild conditions, which decrease the possibility for side processes to take place.[29] The use of the potassium naphthalenide initiator system yielded the copolymerization of AGE and BnGE at room temperature with very few side reactions.

To ensure the water insolubility of the final P(HZ-*co*-BnGE) (**3b**), while maintaining a sufficient number of hydrazide groups per polymer chain for further surface modification, the ratio of comonomers AGE:BnGE was chosen to be 1:2. The copolymer P(AGE-*co*-BnGE) (**1b**) was obtained with high monomer conversions of 99% and 97% for AGE and BnGE, respectively. Its molecular weight estimated by ^{1}H NMR spectroscopy was slightly understated as compared with the theoretical value (Table 1), which is probably due to the overlap of the signals of initiator and main PEG chain. The GPC trace of the polymer **1b** showed a perfectly symmetric peak (Figure S1A, Supporting Information) that together with low polydispersity index (1.07) indicated very few side reactions during polymerization. Therefore, a small variation of the molecular weight of the polymer **1b** from the theoretically estimated value can be attributed to the use of polystyrene calibration system (different structure of polymer), rather than to chain transfer reactions. For comparison, molecular weight of polymer **1a** determined by ^{1}H NMR and GPC analysis was similar to that theoretically calculated. An increased polydispersity index could likely be due to the partial chain coupling at high monomer conversions. Since copolymer **1b** is a viscous oil and cannot be purified by precipitation, as in the case of bifunctional PEGs, chromatographic purification on silica gel is required. As expected, low-molecular-weight impurities were eluted from silica with chloroform. Pure polymer was subsequently isolated by elution with a chloroform/methanol (10:0.7) mixture. NMR and GPC analysis confirmed identical compositions and molecular weight distributions of the crude and purified polymer **1b** (Figure S1A, Supporting Information).

Further conversion of polymer **1b** into the corresponding ester **2b** was performed via thiol-ene reaction with methyl mercapto acetate. The excess thiol was used to prevent the radical-initiated cross-linking of allyl groups. Under these conditions, the reaction proceeded with quantitative conversion that is crucial because the separation of unmodified starting material was not possible. The excess thiol used in the reaction was successfully removed chromatographically, similar to the purification described for polymer **1b**. GPC chromatograms of polymers **1b** and **2b** are shown in Figure S1B (Supporting Information). Purified polymer **2b** was finally reacted with an excess of hydrazine, yielding the desired P(HZ-*co*-BnGE) (**3b**). Complete conversion of the ester groups into

Table 1. Characterization of the polymers obtained by anionic ring-opening polymerization.

	AGE equiv.[a]	BnGE equiv.[a]	$M_{theor.}$ [g mol^{-1}]	NMR[b]		GPC[c]			PDI[e]
				AGE conv.[d]	BnGE conv.[d]	$\overline{M}_n$ [g mol^{-1}]	$\overline{M}_n$ [g mol^{-1}]	$\overline{M}_w$ [g mol^{-1}]	
1a	60	–	6800	99	–	6600	6900	7700	1.11
1b	20	40	8800	99	97	8300	7700	8200	1.07

[a]Number of monomer equivalents used for polymerization; [b]Calculated based on ^{1}H NMR spectra of the polymers (solvent $CDCl_3$); [c]Determined by GPC in THF; [d]Conversion of a monomer; [e]Polydispersity index = $\overline{M}_w/\overline{M}_n$.

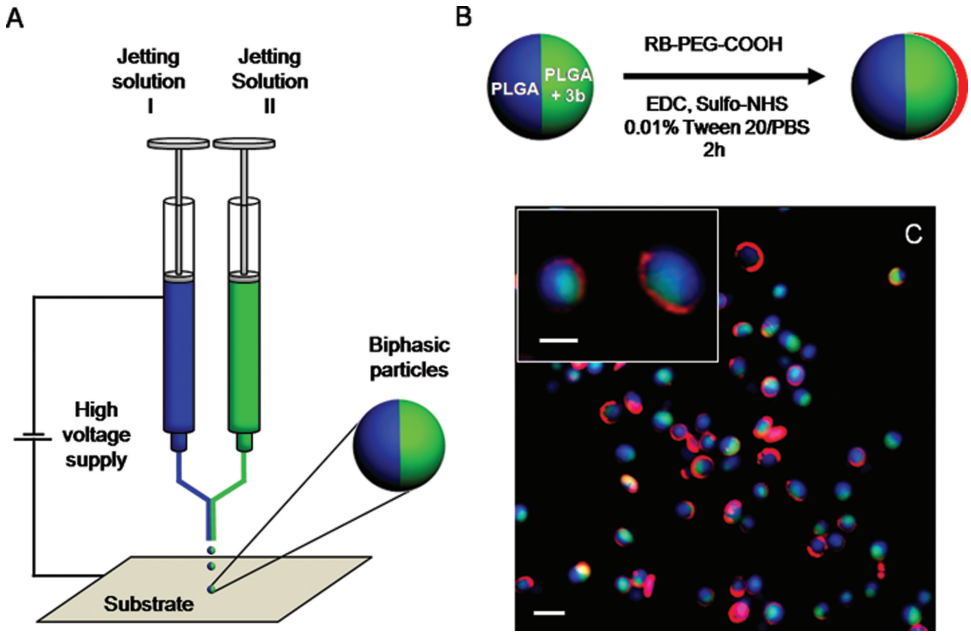


Figure 1. Schematic representation of electrohydrodynamic co-jetting technique for the generation of Janus particles (A). Modification of bicompartmental PLGA/polymer **3b** microspheres with RB-PEG-COOH (B) and CLSM images of these microspheres (C) showing selective binding of RB-PEG-COOH to the compartment containing polymer **3b**. Blue, green, red dyes indicate PLGA, polymer **3b**, and RB-PEG-COOH, respectively. Scale bar: 5 μm (inset 2 μm).

hydrazide groups was indicated by the complete disappearance of the singlet of the methoxy groups in the ^{1}H NMR spectrum of polymer **3b** (Figure S2, Supporting Information). Since polymer **3b** is soluble in organic solvents, simply washing a solution of polymer **3b** in chloroform with water led to removal of excessive hydrazine hydrate. Comparison of MALDI-TOF-MS spectra of polymers **2b** and **3b** revealed no change in the molecular weight (Figure S3, Supporting Information). Moreover, after modifications, the ratio of comonomers in polymer **3b** remained 1:2, as calculated from the ^{1}H NMR spectrum of polymer **3b** (Figure S2, Supporting Information).

The obtained polymer **3b** showed a very low solubility in water, i.e., ≈0.4 μg mL^{-1}. By comparison, the solubility of an analogous homo-multifunctional PHZ (**3a**) (Scheme 1) has been estimated to be equal to 0.5 mg mL^{-1}, which is more than three orders of magnitude higher than that of polymer **3b** (the representative solutions of polymers **3b** and **3a** are shown in Figure S4, Supporting Information).

To investigate potential biomedical uses of these multifunctional polymers, we expanded our work to the development of bicompartmental microspheres and microcylinders in which polymer **3b** was selectively loaded in only one of the two compartments. For this purpose, PLGA/polymer **3b** microspheres were prepared through EHD co-jetting. Two different PLGA solutions with a concentration of 9 w v^{-1}% were prepared in solvent mixtures of chloroform and dimethylformamid (DMF) (95:5, v v^{-1}), and polymer **3b** (0.9 w v^{-1}%, 10% by weight of PLGA) was introduced in one of the solutions. The polymer solutions were flown through side-by-side capillaries in the presence of an appropriate electrical voltage. Microspheres with a diameter of 2–3 μm were prepared. The obtained microspheres were then collected from the jetting collector and the presence of hydrazide groups on the particle surface was confirmed via the conjugation of a fluorescent dye (RB-PEG-COOH) to the particles' surface (Figure 1). By using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) coupling, the carboxylic acid groups of RB-PEG-COOH were activated to further react with the hydrazide groups on the microspheres. In confocal laser scanning microscopy (CLSM) images of the particles after the reaction, the red fluorescence layer from the rhodamine was observed only on the compartment containing polymer **3b** (green), which verified that the hydrazide groups were present only on one compartment. Significantly, this demonstrated the selective localization of polymer **3b** to the predefined structures. Moreover, exploitation of the hydrazide groups of polymer **3b** to further modify the microsphere surface demonstrates potential binding sites for biomolecules.

To validate the concept of using these functionally patterned microstructures for spatially controlled binding of biomolecules, bicompartmental microcylinders containing polymer **3b** in one compartment were prepared and carbohydrates were selectively immobilized. The

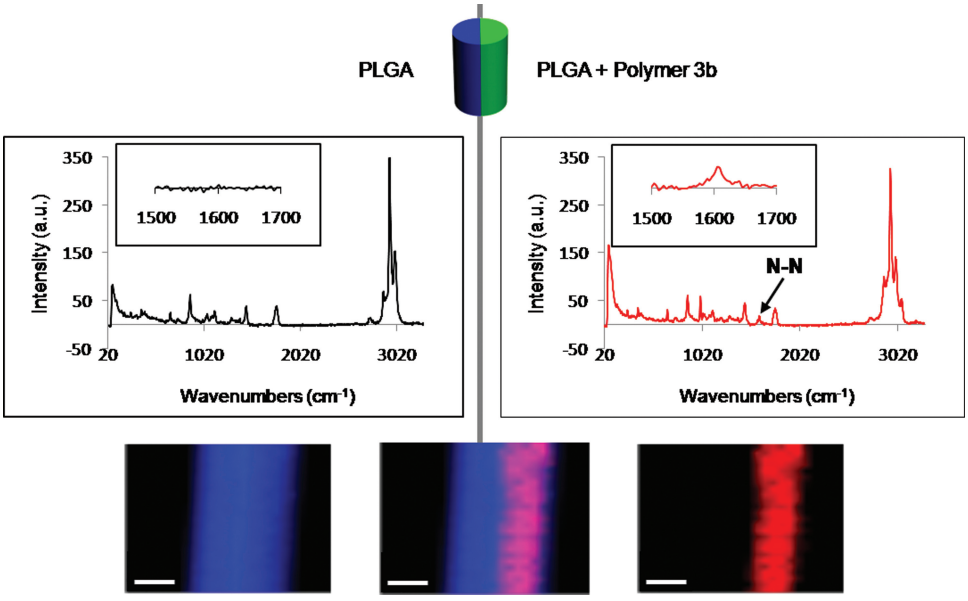


Figure 2. Confocal Raman microscopy images of the bicompartmental PLGA/polymer **3b** microcylinders, showing the localization of polymer **3b** just in one compartment. Scale bars: 5 μm.

microcylinders were produced through the EHD co-jetting method, which was similar to that of the microspheres, except that the concentration of PLGA and polymer **3b** were increased to 30 w v^{-1}% and 15 w v^{-1}% (50% by weight of PLGA), respectively. In addition, a rotary collector was used during EHD co-jetting to acquire highly aligned bundles of microfibers. The microfibers were further processed to create uniform microcylinders by a previously reported microsectioning technique.[30] The selective localization of hydrazide groups on the obtained PLGA/polymer **3b** microcylinders was determined by confocal Raman microscopy (Figure 2). Through subsequent confocal Raman imaging, the hydrazide band at 1600 cm^{-1} was observed, and the confocal image demonstrated that the corresponding spectra were found only in one compartment of the microcylinder.

Finally, the binding affinity of carbohydrates to the PLGA/polymer **3b** microcylinders was examined with 2α-mannobiose, serving as a model carbohydrate. In order to bind 2α-mannobiose to the microcylinders, the sugar was first oxidized through the addition of sodium periodate. The oxidation step was required to obtain free aldehyde groups, which were used for the covalent binding of 2α-mannobiose to the hydrazide groups on the microcylinder hemisphere. Next, the successful binding of 2α-mannobiose to the microcylinders was unambiguously confirmed by introducing carbohydrate-lectin interactions. Lectins are well-known carbohydrate-recognizing proteins, which show reversible and site-specific binding behavior. Thus, the presence of 2α-mannobiose on the microcylinders was determined by adding mannose-specific lectin, concanavalin A (Con A, rhodamine-labeled). After 3 h of incubation of the 2α-mannobiose-bound microcylinders with Con A, the CLSM images of the microcylinders verified the selective binding of 2α-mannobiose to the bicompartmental PLGA/polymer **3b** microcylinders (Figure 3).

3. Conclusions

A novel multifunctional P(HZ-*co*-BnGE) polymer was synthesized via multistep modification of P(AGE-*co*-BnGE), which was prepared for this purpose by anionic ring-opening copolymerization of AGE and BnGE. The polymer showed low water solubility as compared with the homo-multifunctional PHZ. Bicompartmental microspheres and microcylinders with P(HZ-*co*-BnGE) in one of the compartments were then generated via EHD co-jetting. Selective conjugation of RB-PEG-COOH via the use of EDC assisted coupling on one microsphere compartment verified the presence of hydrazide groups on half the microparticle surface. Furthermore, these hydrazide groups were exploited to bind 2α-mannobiose to the PLGA/P(HZ-*co*-BnGE) microcylinders and this immobilization was confirmed by successful carbohydrate-lectin interactions. Fabricated anisotropic microparticles, with reactive hydrazide groups on half the surface, are a promising material for biomedicine. This is particularly true in the area of targeted drug delivery, since the particles readily react with sugars, which in turn are specifically recognized by protein receptors such as lectins and stimulate receptor-mediated endocytosis.

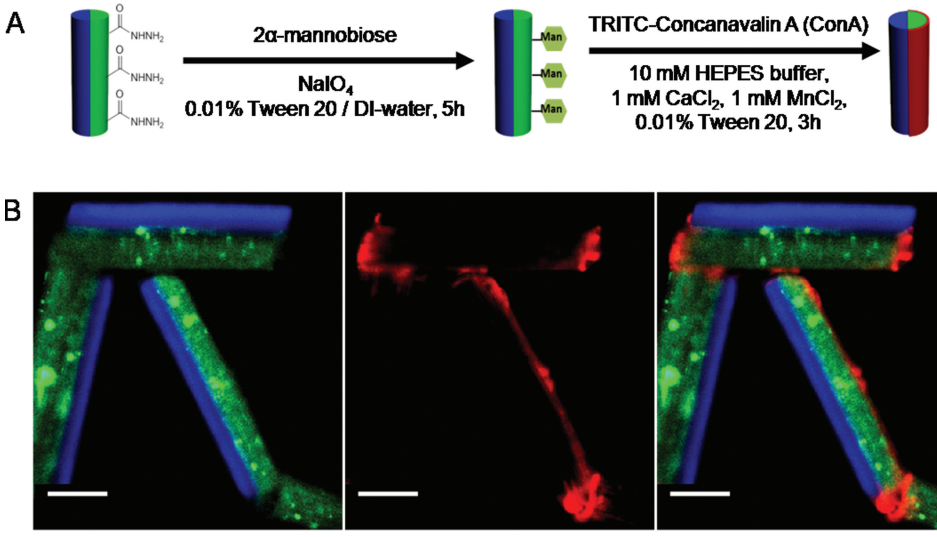


Figure 3. A,B) Reaction scheme and CLSM images showing selective binding of 2α-mannobiose followed by ConA conjugation on the bicompartmental PLGA/polymer **3b** microcylinders. The overlaid CLSM images demonstrate successful immobilization of 2α-mannobiose and ConA selectively on the polymer **3b** compartment. Blue, green, red dyes indicate PLGA, polymer **3b**, and ConA, respectively. Scale bars: 20 µm.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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