Electrospun polymeric short microfibers with surface-selective functionalization

Matsuura, Tetsuya
Tsuchiya, Eiko
Fukui, Yu
Maruyama, Tatsuo

(Citation)
Colloid and Polymer Science,296(1):239-244

(Issue Date)
2018-01

(Resource Type)
journal article

(Version)
Accepted Manuscript

Electrospun polymeric short microfibers with surface-selective functionalization

Tetsuya Matsuura, Eiko Tsuchiya, Yu Fukui, Tatsuo Maruyama*

Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe 657-8501, Japan

***Corresponding Author**

Tel & Fax: +81-78-803-6070

E-mail: tmarutcm@crystal.kobe-u.ac.jp

Abstract

We prepared short microfibers comprising a mixture of a poly(methyl methacrylate)–poly(glycidyl methacrylate) copolymer and polymethylmethacrylate by electrospinning followed by ultrasonication treatment. The resulting short microfibers were 1.5 μm in diameter and 5–30 μm long. We then carried out surface-selective functionalization of the short microfibers using fluorescently labeled proteins. Microscopic observation revealed the lateral surface-selective and cross-section-selective modification of the short microfibers. Finally, we succeeded in the self-organization of the surface-functionalized short microfibers with polymeric microspheres to form complicated microstructures via specific interactions between biomolecules.

Keywords: Electrospinning, Microfibers, Surface functionalization, Anisotropic materials, Mesoscopic structures

Introduction

Functional colloidal particles comprising a variety of substances including polymeric and inorganic materials have been extensively investigated for use in biotechnology [1], nanotechnology [2], electronics [3], and clean/reusable energy [4]. The shapes of colloidal particles often affect their function and use as building blocks. To overcome the intrinsic limitations of isotropic particles, attempts have been made to mimic the anisotropic particles that exist in abundance in nature. The anisotropic particles that occur in nature, such as cells, bacteria, pollen, organelles, etc., have a broad range of excellent functions [5]. Therefore, precise engineering of anisotropy in colloidal particles has recently attracted increased attention, and a number of investigations have focused on the fabrication of non-spherical colloidal particles [6-10]. The development of asymmetric colloidal particles such as rods, disks, fibers, tubes, dumbbell-shaped particles, conical particles, sheets, and ellipsoids has been reported using various fabrication techniques [5,11-22]. There has also been pioneering work on the preparation of anisotropically functionalized colloidal particles [23-27].

Of the various forms of colloidal particles, short microfibers are attractive candidates for anisotropic materials because they have remarkably high aspect ratios and multifaceted architecture. However, to the best of our knowledge there are only a limited number of reports on the preparation

of short microfibers, which has proven to be difficult and suffers from low productivity, complicated procedures or damaging to the polymeric fibers [28-33]. Very few researchers have studied the anisotropic functionalization of short microfibers [28,29]. In the present study, we established a novel method for preparing polymeric short microfibers that involves the ultrasonication treatment of electrospun microfibers.

Ultrasonication treatment has several advantages: it is simple, requires mild conditions, and has high productivity compared with existing methods such as microsectioning [28,29], UV or laser cutting [30,31], and micromolding [32,33]. The present study enabled the surface-selective functionalization of short microfibers. Moreover, the surface-functionalized short microfibers self-organized with polymeric microspheres to form complicated microstructures via avidin–biotin interaction, which demonstrates the potential of the surface functionalization of anisotropic materials for mesoscopic structuring based on molecular recognition. To the best of our knowledge, this is the first approach for preparing short microfibers with surface-selective modification using electrospun microfibers and biomolecules. The present approach for the construction of microstructure using the surface-functionalized short microfibers can be applied to optical device, analytical sensors, fluid handling and electronic wiring in substrates etc.

Materials and Methods

Materials

Methyl methacrylate (MMA), glycidyl methacrylate (GMA), 2,2′-azobis(2,4-dimethylvaleronitrile) (AIBN), toluene, methanol, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and bovine serum albumin (BSA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Polymethylmethacrylate (PMMA, *M*w = 120,000), albumin–fluorescein isothiocyanate conjugate (FITC-BSA), and (+)-biotin hydrazide were purchased from Sigma (St. Louis, MO, USA). NeutrAvidin was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Tetramethylrhodamine isothiocyanate (TRITC) was purchased from Invitrogen (Carlsbad, CA, USA). Biotin-coated polystyrene microspheres (3.3 μm) were purchased from Spherotech (Lake Forest, IL, USA).

Poly(MMA-GMA) copolymer was synthesized from the corresponding monomers (MMA and GMA) by conventional free-radical polymerization using AIBN as an initiator. MMA and GMA were purified by distillation under reduced pressure prior to use. MMA (23.4 ml), GMA (7.5 ml), toluene (52.1 ml), and AIBN (75 mg) were mixed, and polymerization was carried out at 80 °C for 24 h. The reaction mixture was then poured into excess methanol to precipitate the polymer, which was collected by filtration and dried under vacuum.

TRITC-labeled NeutrAvidin was prepared as follows. NeutrAvidin (10 mg) was dissolved in a sodium carbonate buffer (pH 8.8, 0.1 M, 9 ml). A DMSO solution (1 ml) containing 1 mg of TRITC was added to the NeutrAvidin solution. After 24 h at 4 °C, the reaction solution was dialyzed with an excess amount of water using a dialysis membrane (MWCO 15 kDa) overnight, followed by

freeze-drying.

Preparation of polymeric short microfibers

The electrospinning (NF-102, MECC Co., Ogori, Japan) experimental equipment consisted of a syringe pump, a stainless-steel needle, and a high-voltage generator [34, 35]. Typically, a needle was loaded with a DMF solution containing 24 wt% poly(MMA-GMA) and 6 wt% PMMA. The solution was electrospun from the needle (cathode), into an oppositely charged stainless-steel dish (anode). The feed rate for the solution was set at 0.2 ml/h, and the working voltage was 24 kV. The distance from the needle to the collector was 23 cm. The inner and outer diameters of the needle were 330 μm and 630 μm, respectively.

After electrospinning, the resulting microfibers were immersed in 0.1 mg/ml BSA solution in phosphate buffer (pH 7.0, 50 mM) to immobilize BSA on the surfaces of the microfibers, and then immersed in Tris-HCl buffer (pH 7.0, 50 mM) to wash the microfibers. The immersed microfibers were cut into 2–3 mm lengths using a razor, and sonicated in phosphate-buffered saline (PBS, pH 7.4) for 3 h using an ultrasonication bath (VS-D100, Asone, Osaka, Japan) to prepare short microfibers. One hour after ultrasonication, the crude long microfibers were removed by filtration using a nylon net filter (pore size 11 μm) (Merck Millipore, Darmstadt, Germany) to obtain short microfibers. The resulting short microfibers were observed using an inverted microscope (IX71, Olympus Co., Tokyo, Japan).

Surface-selective modification of polymeric short microfibers

Short microfiber 1:

Microfibers comprising poly(MMA-GMA) and PMMA were prepared by electrospinning, as described above. After electrospinning, the microfibers were immersed in phosphate buffer (pH 7.0, 50 mM) containing 0.1 mg/ml FITC-BSA to immobilize FITC-BSA on the lateral surfaces of the microfibers. The microfibers were then washed by immersing them in Tris-HCl buffer (pH 7.0, 50 mM). The immersed microfibers were cut into 2–3 mm lengths using a razor. The cut microfibers were sonicated in sodium carbonate buffer (pH 9.5, 50 mM) containing 1 mM biotin hydrazide for 3 h to prepare short microfibers with biotin hydrazide-modified cross-sections. One hour after sonication, the crude long microfibers were removed by filtration using a nylon net filter (pore size 11 μm) (Millipore, Darmstadt, Germany). After washing repeatedly with water, the short microfibers were immersed in PBS containing 0.1 mg/ml TRITC-NeutrAvidin to immobilize TRITC-NeutrAvidin at the cross-sections. After collecting the short microfibers using a nylon net filter (pore size 11 μm), the resulting short microfibers were washed repeatedly with water, and observed using a confocal laser scanning microscope (CLSM) (FV1000-KDM, Olympus Co., Tokyo, Japan).

Short microfiber 2:

Microfibers with NeutrAvidin-modified lateral surfaces were prepared in a similar way to that described above using phosphate buffer (pH 7.0, 50 mM) containing 0.1 mg/ml NeutrAvidin. The microfibers were cut using a razor and sonicated in PBS (pH 7.4) for 3 h to prepare short microfibers with NeutrAvidin-modified lateral surfaces. They were then collected using a nylon net filter (pore size 11 μm).

Short microfiber 3:

Microfibers with BSA-modified lateral surfaces were prepared in a similar way to that described above using phosphate buffer (pH 7.0, 50 mM) containing 0.1 mg/ml BSA. The microfibers were cut using a razor and sonicated in PBS (pH 7.4) for 3 h to prepare short microfibers without NeutrAvidin-modified lateral surfaces. They were then collected using a nylon net filter (pore size 11 μm).

Self-assembly of short microfibers and microspheres

Each short microfiber was immersed in a PBS suspension (pH 7.4) containing 100 μl/ml biotinylated polystyrene microspheres overnight. The resulting microstructures were observed using an inverted microscope and a field emission scanning electron microscopy (FE-SEM). The samples were sputter-coated with osmium (Os) prior to observation by FE-SEM.

Results and Discussion

We subjected a mixed solution of poly(MMA-GMA) and PMMA to electrospinning to align microfibers of approximately 1.5 μm diameter, as shown in Fig. 1a. The fiber morphology can be controlled by spinning parameters such as the concentration and solvent of spinning a polymer solution, voltage, needle diameter and spinning distances as previously reviewed [36]. In the present study, the orientation of electrospun microfibers was controlled using a rotating disk as a collector of the fibers. Fig. 1b shows that short microfibers (5–30 μm long) were successfully prepared by ultrasonication. Robust and low-elastic mechanical properties of a PMMA-based polymer would help the cutting induced by ultrasonication. We did not observe any long fibers (> 100 μm long) after filtration. These results demonstrate that ultrasonication treatment followed by filtration is an effective technique for preparing short PMMA-based microfibers with a relatively narrow length distribution.

Surface-selective functionalization is of large importance for micro- and nano-structured anisotropic materials. We investigated the surface-selective functionalization of short microfibers using fluorophore-labeled proteins (*Short microfiber 1*). The glycidyl groups in poly(MMA-GMA) can react with the amino groups in proteins and the hydrophobicity of a PMMA-based surface helps adsorption of proteins to its surface [37]. As shown in Fig. 2a, we first immobilized FITC-BSA on the lateral surfaces of the microfibers by exploiting the glycidyl groups and the hydrophobicity of the surfaces of the electrospun microfibers to cover the surface of PMMA to prevent the subsequent

undesired adsorption and interaction to the surface, and blocked the remaining glycidyl groups using tris(hydroxymethyl)aminomethane (Tris base). While cutting the electrospun microfibers to short microfibers by ultrasonication, we covalently modified the cross-sections of the short microfibers with biotin hydrazide using the glycidyl groups at the cross-sections. We then added TRITC-NeutrAvidin to selectively modify the cross-sections of the short microfibers. CLSM observation revealed green fluorescence at the lateral surfaces of the short microfibers, which was derived from FITC-BSA. And red fluorescence was found only at the cross-sections, indicating the selective immobilization of TRITC-NeutrAvidin (Fig. 2b). This demonstrates the successful surface-selective modification of the short microfibers using avidin-biotin interaction.

Surface-selectively functionalized anisotropic materials have the potential to create complicated three-dimensional architectures by self-organization. Here, we investigated the self-organization of microstructures consisting of polymeric short microfibers and microspheres using avidin–biotin interaction (Fig. 3a). The cross-sections or lateral surfaces of the short microfibers were selectively modified with NeutrAvidin using the procedure described above (*Short microfibers 1 and 2*), and the short microfibers without NeutrAvidin-modified surfaces were also prepared as *Short microfiber 3*. The short microfibers were mixed with the biotinylated microspheres. Fig. 3b and e shows bright field microscopy and FE-SEM images of the microstructure consisting of biotinylated microspheres and short microfibers with NeutrAvidin-modified cross-sections. As illustrated in Fig. 3a, we observed dumbbell-shaped microstructures, which were formed by the specific adhesion of the

microspheres to the cross-sections of the short microfibers (*Short microfiber 1*) due to avidin–biotin interaction. Seventy % of the cross-sections of the short microfibers were adhered with the microspheres. Notably, the microspheres did not adhere to the lateral surfaces of the short microfibers, which suggests that NeutrAvidin was absent at the lateral surfaces of the short microfibers and also indicates the successful surface-selective modification.

We observed grape-shaped microstructures when the short microfibers with NeutrAvidin-modified lateral surfaces (*Short microfiber 2*) were used (Fig. 3c and f). It should be noted that the short microfibers without NeutrAvidin-modified surfaces (*Short microfiber 3*) did not interact with the biotinylated microspheres, and did not form complexes (Fig. 3d). Free microspheres were observed in Fig. 3b and c because a relatively excess amount of microspheres were added to the microfibers. These results prove that the high-order microstructures were organized via a specific interaction between NeutrAvidin and biotin on the selectively functionalized surfaces of the short microfibers and microspheres. They also suggest that molecular interactions at nano scale between a protein and a small molecule can be utilized for the assembly of substrates at micro scale.

Conclusions

We successfully prepared short polymeric microfibers using a novel, simple, and efficient method comprising the ultrasonication treatment of electrospun microfibers. Moreover, the present method enabled surface-selective functionalization of the short microfibers. Finally, complicated

microstructures were created by the self-organization of the surface-selectively functionalized short microfibers and microspheres. The findings of the present study suggest that electrospun short microfibers have great potential as micro-structured anisotropic materials for surface-selective functionalization and can be applied to the mesoscopic-scale construction of new architectures by means of specific molecular recognition.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This study was financially supported by the Special Coordination Funds for Promoting Science and Technology, Creation of Innovation Centers for Advanced Interdisciplinary Research Areas (Innovative Bioproduction Kobe), MEXT, Japan, and by JSPS KAKENHI Grant Numbers 16H04577 & 16K14491.

References

1. Nagarwal RC, Kant S, Singh PN, Maiti P, Pandit JK (2009) Polymeric nanoparticulate system: a potential approach for ocular drug delivery. J Control Release 136:2–13

2. Grosse C, Delgado AV (2010) Dielectric dispersion in aqueous colloidal systems. Curr Opin Colloid Interface Sci 15:145–159

3. Wood V, Panzer MJ, Caruge JM, Halpert JE, Bawendi MG, Bulović V (2010) Air-stable operation of transparent, colloidal quantum dot based LEDs with a unipolar device architecture. Nano Lett 10: 24–29

4. Rhodes R, Asghar S, Krakow R, Horie M, Wang Z, Turner ML et al (2009) Hybrid polymer solar cells: From the role colloid science could play in bringing deployment closer to a study of factors affecting the stability of non-aqueous ZnO dispersions. Colloids Surf A 343:50–56

5. Mitragotri S, Lahann J (2009) Physical approaches to biomaterial design. Nat Mater 8:15–23

6. Yoo JW, Doshi N, Mitragotri S (2010) Endocytosis and intracellular distribution of PLGA particles in endothelial cells: effect of particle geometry. Macromol Rapid Commun 31:142–148

7. Decuzzi P, Ferrari M (2008) The receptor-mediated endocytosis of non-spherical particles. Biophys J 94:3790–3797

8. Shum HC, Abate AR, Lee D, Studart AR, Wang B, Chen CH et al (2010) Dropletmicrofluidics for fabrication of non-spherical particles. Macromol Rapid Commun 31:108–118

9. Misra A, Urban MW (2010) Acorn-shape polymeric nano-colloids: synthesis and self-assembled films. Macromol Rapid Commun 31:119–127

10. Haghgooie R, Toner M, Doyle PS (2010) Squishy non-spherical hydrogel microparticles. Macromol Rapid Commun 31:128–134

11. Perro A, Reculusa S, Ravaine S, Bourgeat-Lami E, Duguet E (2005) Design and synthesis of Janus micro-and nanoparticles. J Mater Chem 15:3745–3760

12. Walther A, Muller AHE (2008) Janus particles. Soft Matter 4:663–668

13. Wurm F, Kilbinger AFM (2009) Polymeric Janus particles. Angew Chem Int Ed 48:8412–8421

14. Yang SM, Kim SH, Lim JM, Yi GR (2008) Synthesis and assembly of structured colloidal particles. J Mater Chem 18:2177–2190

15. Jiang S, Chen Q, Tripathy M, Luijten E, Schweizer KS, Granick S (2010) Janus particle synthesis and assembly. Adv Mater 22:1060–1071

16. Pawar AB, Kretzschmar I (2010) Fabrication, assembly, and application of patchy particles. Macromol Rapid Commun 31:150–168

17. Dendukuri D, Doyle PS (2009) The synthesis and assembly of polymeric microparticles using microfluidics. Adv Mater 21:4071–4086

18. Glotzer SC, Solomon MJ (2007) Anisotropy of building blocks and their assembly into complex structures. Nat Mater 6:557–562

19. Yoon J, Jung YS, Kim JM (2009) A combinatorial approach for colorimetric differentiation of organic solvents based on conjugated polymer-embedded electrospun fibers. Adv Funct Mater 19:209–214

20. Lee KJ, Oh WK, Song J, Kim S, Lee J, Jang J (2010) Photoluminescent polymer nanoparticles for label-free cellular imaging. Chem Commun 46:5229–5231

21. Hong JY, Kwon E, Jang J (2009) Fabrication of silica/polythiophene core/shell nanospheres and their electrorheological fluid application. Soft Matter 5:951–953

22. Jang J (2006) Conducting polymer nanomaterials and their applications. Adv Polym Sci 199:189–259

23. Nie Z, Li W, Seo M, Xu S, Kumacheva E (2006) Janus and ternary particles generated by microfluidic synthesis: design, synthesis, and self-assembly. J Am Chem Soc 128:9408–9412

24. Dendukuri D, Pregibon DC, Collins J, Hatton TA, Patrick SD (2006) Continuous-flow lithography for high-throughput microparticle synthesis. Nat Mater 5:365–369

25. Dendukuri D, Hatton TA, Patrick SD (2007) Synthesis and self-assembly of amphiphilic polymeric microparticles. Langmuir 23:4669–4674

26. Bong KW, Bong KT, Pregibon DC, Doyle PS (2010) Hydrodynamic focusing lithography. Angew Chem Int Ed 49:87–90

27. Tan WS, Lewis CL, Horelik NE, Pregibon DC, Doyle PS, Yi H (2008) Hierarchical assembly of viral nanotemplates with encoded microparticles via nucleic acid hybridization. Langmuir 24:12483–12488

28. Srijanani Bhaskar, Jonathon Hitt, Sei-Won Laura Chang, Joerg Lahann (2009) Multicompartmental microcylinders. Angew Chem Int Ed 48:4589–4593

29. Sampa Saha, Davor Copic, Srijanani Bhaskar, Nicholas Clay, Alessandro Donini, A. John Hart, Joerg Lahann (2012) Chemically controlled bending of compositionally anisotropic microcylinders. Angew Chem Int Ed 51:660–665

30. Aleksandar Stoiljkovic, Seema Agarwal (2008) Short electrospun fibers by UV cutting method.

Macromol Mater Eng 293:895–899

31. Olaf Kriha, Mathias Becker, Martina Lehmann, Dorothee Kriha, Josef Krieglstein, Maekele Yosef, Sabine Schlecht, Ralf B. Wehrspohn, Joachim H. Wendorff, Andreas Greiner (2007) Connection of hippocampal neurons by magnetically controlled movement of short electrospun polymer fibers – a route to magnetic micromanipulators. Adv Mater 19:2483–2485

32. Jie-Yu Wang, Yapei Wang, Sergei S. Sheiko, Douglas E. Betts, Joseph M. DeSimone (2012) Tuning multiphase amphiphilic rods to direct self-assembly. J Am Chem Soc 134:5801–5806

33. Olaf Kriha, Petra Göring, Marc Milbradt, Seema Agarwal, Martin Steinhart, Ralf Wehrspohn, Joachim H. Wendorff, Andreas Greiner (2008) Polymer tubes with longitudinal composition gradient by face-to-face wetting. Chem Mater 20:1076–1081

34. Yu Fukui, Tatsuo Maruyama, Yuko Iwamatsu, Akihiro Fujii, Tsutomu Tanaka, Yoshikage Ohmukai, Hideto Matsuyama (2010) Preparation of monodispersed polyelectrolyte microcapsules with high encapsulation efficiency by an electrospray technique. Colloids Surf A, 370:28-34.

35. Tetsuya Matsuura, Tatsuo Maruyama (2017) Hollow phosphorylcholine polymer vesicles prepared by a coaxial electrospray technique. Colloid Polym Sci 295:1251-1256.

36. NandanaBhardwaj, Subhas C. Kundu (2010) Electrospinning: A fascinating fiber fabrication technique. Biotechnol Adv 28: 325-347

37. Shunsuke Yamamoto, Shigeru Kitahata, Ayane Shimomura, Kaya Tokuda, Takashi Nishino, Tatsuo Maruyama (2015) Surfactant-induced polymer segregation to produce anti-fouling surfaces

via dip-coating with an amphiphilic polymer. Langmuir 31:125-131

Figures

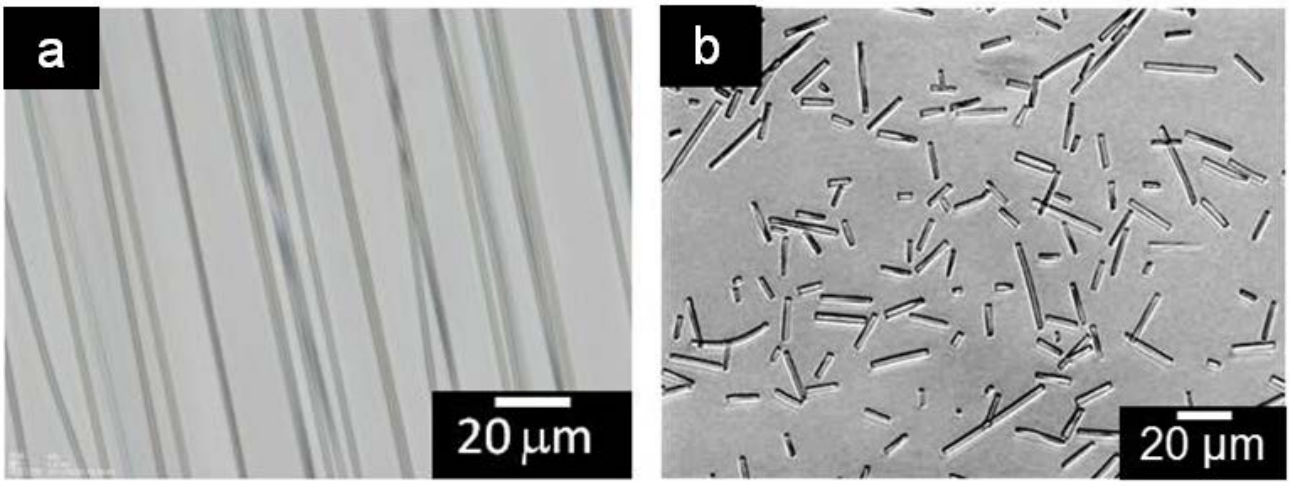

Fig. 1. Bright-field microscope images of (a) electrospun polymeric microfibers and (b) polymeric short microfibers prepared by ultrasonication of electrospun polymeric microfibers.

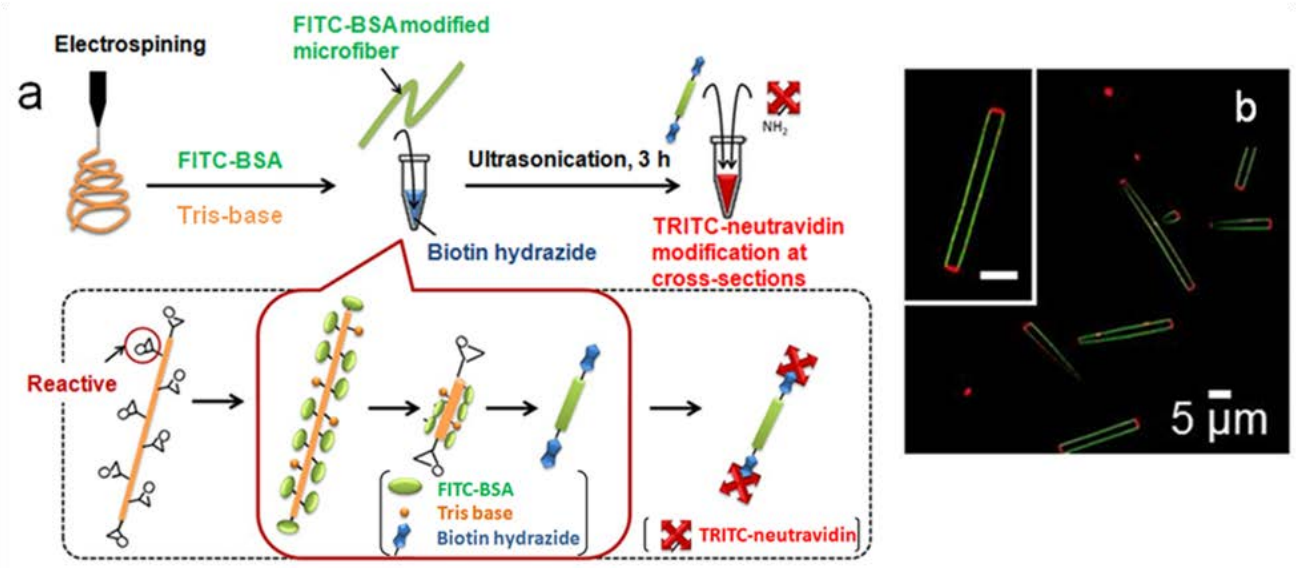

Fig. 2. (a) Schematic of surface-selective modification of polymeric short microfibers. (b) CLSM image of polymeric short microfibers following surface-selective modification. Red and green fluorescence was derived from TRITC-NeutrAvidin and FITC-BSA, respectively.

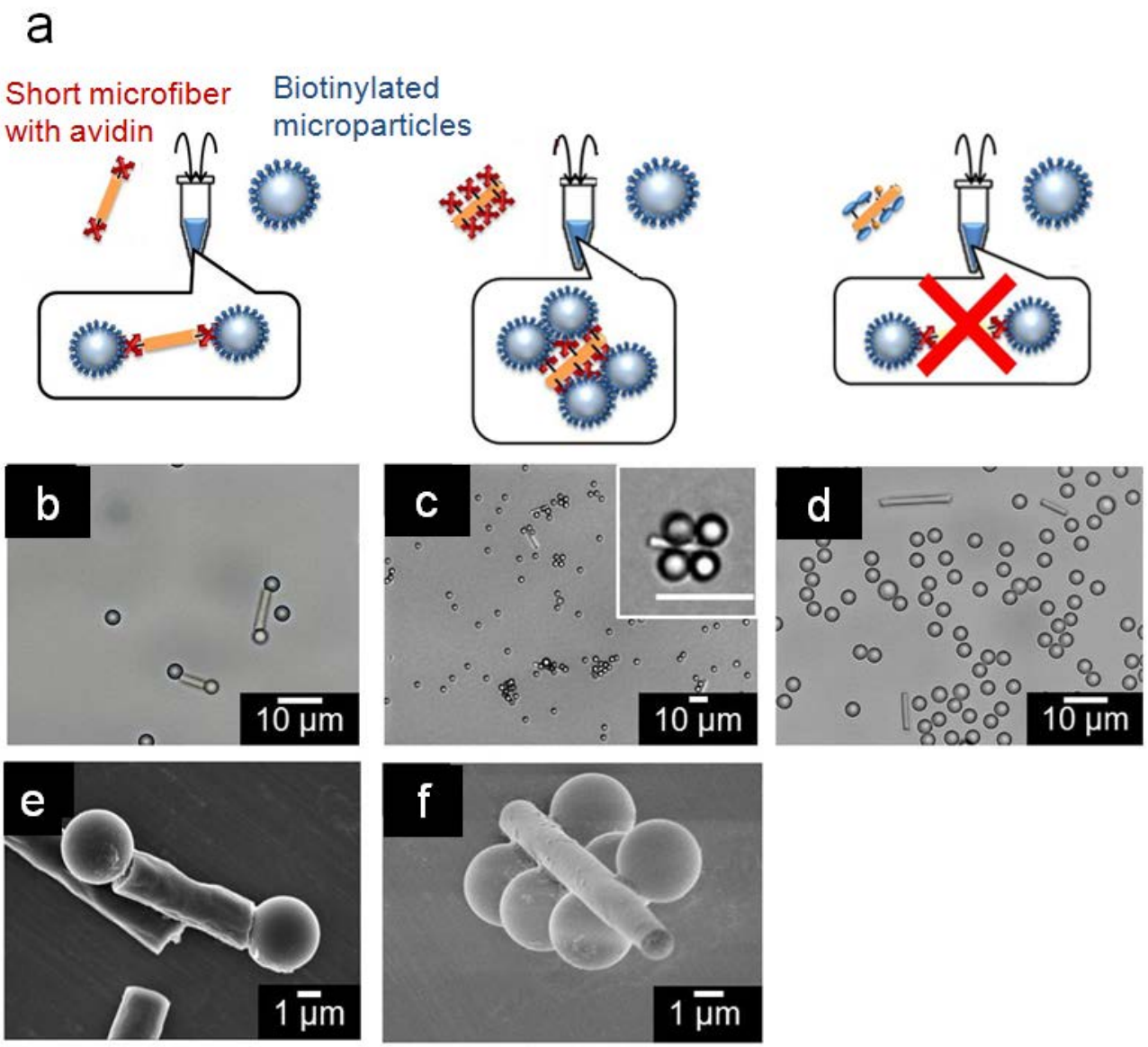

Fig. 3. (a) Schematic of self-organization of polymeric short microfibers with polymeric microspheres. (b, c, d) Bright field microscope images and (e, f) FE-SEM images of microstructures consisting of biotinylated polymeric microspheres and polymeric short microfibers. (b, e) Short microfibers with NeutrAvidin-modified cross-sections. (c, f) Short microfibers with NeutrAvidin-modified lateral surfaces. (d) Short microfibers without NeutrAvidin-modification.