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A general approach to controlled alignment of filamentous supra-biomolecular assemblies into centimeter-scale highly-ordered patterns through nature-inspired magnetic guidance

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Abstract

We took the advantage of the capability of magnetic nanoparticles (MNPs) being aligned along a magnetic field and reproducibly generated large scale bio-nanofiber assemblies with the orientation of the constituent bio-nanofibers defined by the applied magnetic field. When decorated by MNPs, bio-nanofibers could be guided by the external magnetic field to become oriented either horizontally or vertically, forming single- and multi-orientation layered assemblies.

Keywords

Bio-nanofibers; Magnetic nanoparticles; Large scale assemblies; Controlled orientation; Magnetism

Filamentous supra-biomolecular assemblies, such as bacterial flagellum,^[1] bacterial pilus,^[2] and bacteriophage (also called phage),^[3] are naturally occurring bio-nanofibers assembled from their subunits in a highly ordered fashion. Due to the structural ordering, chemical and thermal stability, and genetically tunable surface chemistry, bio-nanofibers can be used as templates for directing the synthesis and assembly of functional nanomaterials such as bone minerals^[4] and silica^[5]. However, to assemble bio-nanofibers into controlled structures at a large scale (e.g., up to centimeter scale) is still a daunting challenge. Currently, several methods have been tried to form assemblies of bio-nanofibers, including electrostatic interaction,^[4a] liquid-crystalline assembly,^[6] capillary forces,^[7] molecular recognitions.^[2a] However, these methods are relatively complicated in handling and most of them (except liquid-crystalline assembly method) can only generate small scale nanostructures. In addition, although large scale vertical assemblies of filamentous inorganic hard nanostructures such as nanotubes of boron carbonitride,^[8] carbon,^[9] titania nanotubes,^[10] and nanowires of GaAs^[11], Si^[12], ZnO^[13], cadmium chalcogenide^[14], have been reported, to the best of our knowledge, there has never been a reported method for the large scale vertical assembly of filamentous soft bio-nanofibers. To advance the practical use of bio-nanofibers with various shapes and functionalities in building devices, it is important to have a facile method that can lead to either single- or multi-layered assemblies with controlled (either horizontal or vertical) orientation of constituent bio-nanofibers on a solid substrate.

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Therefore, an easy-handling method is needed to assemble the bio-nanofibers into large scale assemblies with controlled orientation.

In nature, Fe_3O_4 is present in many organisms such as magnetotactic bacteria (MTB), honey bees, pigeons, dolphins, and butterflies,^[15] allowing the Earth's magnetic dipolar field to assist their orientation, navigation or homing. MTB, as one of the most well-known examples, are capable of taking up Fe sources and converting them into magnetic nanoparticles (MNPs) such as Fe_3O_4 or Fe_3S_4 *in vivo*.^[16] As a result, MTB become oriented along the magnetic field lines of the Earth's magnetic field simply because they contain MNPs.^[16] The magnetically guided orientation of organisms such as MTB simply due to their association with MNPs inspired us to use magnetic field to guide the assembly of bio-nanofibers with the help of MNPs (Fig. 1a). Although the arrangement of synthetic MNPs along magnetic lines was applied to align MNPs themselves^[17], carbon nanotubes^[18], and polymers^[19], using them to guide the alignment of biological nanofibers into long-range-ordered patterns has never been reported. Here, we report the controlled alignment of three structurally different types of bio-nanofibers, including wave-like bacterial flagellar filaments (termed flagella for convenience), straight type 1 bacterial pili, and semi-flexible M13 phage, which were first decorated by Fe_3O_4 MNPs, into long-range-ordered arrays by simply varying the direction and strength of the external magnetic field. Single-orientation arrays (Fig. 1b), where bio-nanofibers were parallel to each other, were fabricated by applying a magnetic field with its direction parallel to the substrates. Multi-orientation multi-layered bio-nanofiber assemblies were synthesized through a layer-by-layer deposition with the magnetic field alternatively twisted for each alternating layer. During the formation of such assemblies, bio-nanofibers were parallel-aligned into one single-orientation layer along one magnetic field and then the magnetic field was twisted into another direction to align bio-nanofibers into a neighboring single-orientation layer (Fig. 1c). In the multi-orientation multi-layered assemblies, the orientation of bio-nanofibers in each layer was parallel to the external magnetic field (Fig. 1c). When the magnetic field was twisted during the deposition of bio-nanofibers, a new neighboring layer made of bio-nanofibers parallel aligned along a different orientation was formed. Vertically aligned bio-nanofiber arrays (Fig. 1d) could also be made by simply changing the external magnetic field to be perpendicular to the substrates. The degrees of ordering of the formed bio-nanofiber arrays were also found to be affected by the strength of external magnetic fields (Fig. S1).

It is known that most proteins and protein-based supra-biomolecular assemblies are negatively charged with an isoelectric point (pI) lower than 7,^[20] including the three filamentous supra-biomolecular assemblies studied in this work, namely, bacterial flagellum (Fig. 1e), type 1 bacterial pilus (Fig. 1f), and M13 phage (Fig. 1g). Flagellar filament is a wave-like nano-filament (14 nm wide and length tunable depending on the number of protein subunits) assembled from several thousand copies of protein subunits called flagellin (Fig. S2a). The solvent-exposed domain of wild-type flagellin (FliC gene product) for bacteria contains negatively charged amino acids such as D and E with a pI estimated to be ~5.3.^[21] Type 1 bacterial pilus is a rigid hair-like appendage (6 nm wide and 1–2 μm long) (Fig. S2c) found on the surface of bacteria. It is predominantly assembled from the protein subunit, FimA, and has a pI of ~3.9 at room temperature.^[2] M13 phage (~6.6 nm wide and 880 nm long), a semi-flexible bio-nanofiber (Fig. S2d), is composed of circular single stranded DNA (ssDNA) encapsulated in approximately 2700 copies of its major coat protein and capped with a few copies of minor coat proteins at both tips. The high copy numbers of solvent-exposed negatively charged amino acids in the N terminus of major coat proteins make M13 phage negatively charged with a pI of ~4.2.^[3, 5, 20] Since most bio-nanofibers are negatively charged, positively charged Fe_3O_4 MNPs were used to electrostatically interact with them to achieve the assembly (and the consequent decoration) of MNPs along them, forming MNP-bio-nanofiber complexes for magnetic-field-guided alignment. On the other

hand, if the surface of a bio-nanofiber changes to positively charged due to genetic engineering,^[5] negatively charged Fe₃O₄ MNPs can also be synthesized to decorate the bio-nanofiber for the alignment. In this work, we used negatively charged bacterial flagellum, type 1 bacterial pilus, and M13 phage as the examples for the study of the alignment of MNP-decorated bio-nanofibers by external magnetic fields. The flagellum, pilus and phage bio-nanofibers were biologically produced and amplified following our reported procedures.^[2a, 21–22]

Positively charged Fe₃O₄ MNPs were synthesized following a reported method.^[23] Briefly, a mixed solution of FeCl₂ (4 mL, 1 M) and FeCl₃ (1 mL, 2 M, in 2 M HCl) was added to an ammonia solution (50 mL, 0.7 M). The black precipitates were collected by centrifugation (10 min at 7500 g), washed with 2 M nitric acid and re-suspended in water to form a transparent and uniform Fe₃O₄ MNP solution. The synthesized MNPs were confirmed by the electron diffraction pattern (Fig. S3a inset)^[24] and their size was about 5–8 nm in diameter (Fig. 2a). The measured room temperature hysteresis curves (Fig. S3 b&c) show that the saturation magnetization and coercivity value of the as-synthesized MNPs are 35 emu/g and 8 Oe, respectively. When Fe₃O₄ nanoparticle solution was placed close to a magnet, Fe₃O₄ nanoparticles were attracted by the magnet (Fig. 2a inset), which also confirmed the superparamagnetism of the synthesized Fe₃O₄ nanoparticles. The as-synthesized MNP solution (70 mg/ml, pH=4.0) was used directly in this work.

In order to verify the interaction between positively charged Fe₃O₄ MNPs and negatively charged bio-nanofibers, they were mixed in an aqueous solution. Immediately, some rust red precipitates were formed (Fig. S4) due to the coating of MNPs onto bio-nanofiber templates. Then the rust red precipitates were observed under a transmission electron microscope (TEM) and were found to be made of MNP-bio-nanofiber complexes (Figs. 2b–d), in which Fe₃O₄ MNPs were assembled along bio-nanofibers (flagella (Fig. 2b), pili (Fig. 2c), or phage (Fig. 2d)) to form a “beads on rods” structure. This fact indicates that our synthesized positively charged Fe₃O₄ MNPs can interact with negatively charged bio-nanofibers through electrostatic forces to form MNP-decorated bio-nanofibers.

To achieve single-orientation horizontally aligned bio-nanofiber arrays (Fig. 1b), Fe₃O₄ MNPs solution (70 mg/ml, pH=4) was mixed with bio-nanofiber solution (flagellum solution, OD_{280nm}=0.1; pilus solution, OD_{280nm}=0.25; phage solution, 10¹³ pfu/mL; pfu: plaque forming unit; all bio-nanofibers dissolved in water with pH=7.0) at a volume ratio of 1/49 (MNP/bio-nanofiber) to form MNP-bio-nanofiber complexes, respectively, and then air-dried on a cover-slide in a constant magnetic field (1 T) with a direction parallel to the surface of the cover-slide (Fig. 1b). The size of assembled MNP-bio-nanofiber structures could reach up to centimeter scale (Fig. S5). The scanning electron microscopy (SEM) and optical microscopy results (Fig. 3) showed that not only Fe₃O₄ MNPs (Fig. 3a) but also MNP-bio-nanofiber complexes (flagellum, Figs. 3b & S6a; pilus, Figs. 3c & S6c; phage, Figs. 3d & S6e) could be parallel aligned along the direction of the magnetic field perfectly. However, when not decorated by Fe₃O₄ MNPs, bio-nanofibers were randomly oriented and not assembled into any aligned pattern even in the presence of a magnetic field (Fig. S7). This fact confirmed that the alignment of bio-nanofibers was due to the alignment of their associated Fe₃O₄ MNPs guided by the magnetic fields. It should be noted that some bio-nanofibers are flexible and curved in their natural form,^[4a, 21a, 22] such as flagella (Figs. 2b, S2b, & S8) and phage (Fig. 2d & S2c). But when they were decorated with MNPs and placed in magnetic fields, they became straight (Fig. 3), indicating the straightening of bio-nanofibers due to the alignment of associated Fe₃O₄ MNPs by magnetic fields. In addition, we used the flagellum-MNP system to systemically study the factors that may affect the bio-nanofiber assembly, including the strength of magnetic field, surface charge of bio-nanofibers, concentration of bio-nanofibers and MNPs, and drying speed. These studies

show that both the strength of magnetic field (Fig. S1) and flagellum surface charges (Fig. S9) greatly affect the ordering of the bio-nanofiber arrays. Only applying a relatively strong magnetic field (1 T) (Fig. S1a) and using flagella that have overall surface charge opposite than the MNPs (i.e., slightly negatively charged WT flagella or more negatively charged flagella displaying peptide EEEEEEEE) (Figs. S9 a&b) can result in highly ordered arrays. The effects of concentrations of bio-nanofibers (Figs. S10&S11) and MNPs (Figs. S12&S13) are trivial once optical density at 280 nm absorption wavelength (OD_{280nm}) of flagella and the mass concentration of MNPs are above 0.01 and 0.14 mg/ml, respectively. In addition, the drying speed, either fast N_2 blow drying or slow air drying (Fig. S14), does not show any effect on bio-nanofiber assembly.

When the direction of a magnetic field was twisted every time after the deposition of a single-orientation layer under the guidance of a magnetic field with the same strength but in a different direction, a multi-orientation multi-layered bio-nanofiber assemblies could be synthesized, where each layer is made of single-oriented bio-nanofibers with orientation defined by the twisting magnetic field (Figs. 1c and 4). Briefly, after the complete drying of the first layer of aligned bio-nanofiber arrays in the presence of a magnetic field, a mixed solution of Fe_3O_4 MNPs and nanofibers were applied onto the first layer and air-dried in a twisted external magnetic field (Fig. 1c). Optical microscopy (Figs. 4a–c & S6b, d, &f) images showed that the bio-nanofiber assemblies were composed of two layers of bio-nanofiber arrays and each layer was made of parallel bio-nanofibers with its direction along the external magnetic field applied during the formation of this layer. By using the magnetically guided layer-by-layer deposition method, it is possible to construct large-scale multi-orientation multi-layered bio-nanofiber structures with a controlled twisting angle between two neighboring layers, which is dictated by the angle between the magnetic fields applied during the deposition of these two layers. Since the direction of each single-orientation layer of bio-nanofibers is determined by the applied magnetic field, the final structure of the multi-orientation multilayered bio-nanofiber assemblies can be easily controlled by controlling the applied magnetic field.

Although self-assembled peptide nanotubes^[25] and rod-like tobacco mosaic virus (TMV)^[26] were vertically aligned before by evaporation and interaction between cysteine and metal substrate, respectively, those methods were limited to rigid peptide nanotubes and TMV, and could not be extended to other bio-nanofibers, especially those long, flexible (e.g., flagellum) or semi-flexible (e.g., M13 phage) bio-nanofibers. In addition, only certain peptides can form nanotubes and the vertical assembly of TMV relies on the presence of cysteine and the use of metal substrate, thus those techniques are limited to biomolecules with certain sequences and/or substrates of certain types. We found that bio-nanofibers could also be vertically aligned on a substrate by simply allowing the direction of the external magnetic field to be perpendicular to the substrate. Towards this end, Fe_3O_4 MNPs solution (70 mg/ml) was mixed with flagellum solution ($OD_{280nm}=1.1$) at a volume ratio of 1/100 and air-dried on a cover-slide in a constant magnetic field (2 T) with a direction perpendicular to the cover-slide (Fig. 1d). SEM imaging (Fig. S15) showed that thousands of flagellum-MNP complex nanofibers were standing on the substrate side-by-side along the direction of magnetic field. We did not observe such vertical alignment when either the magnetic field strength or flagellum concentration was reduced (Figs. S15 b&c), suggesting both strong magnetic fields (2 T) and high concentrations of bio-nanofiber solution (flagellum concentration, $OD_{280nm}=1$) are critical to achieve such vertical alignment of bio-nanofibers. We also found that the type of substrate does not affect assembly. The vertical assembly of filamentous hard inorganic nanostructures on a substrate has been used to develop electronic and magnetic devices, biosensors, and medical implants.^[27] We envision that the vertical assembly of bio-nanofibers could lead to novel bio-based structures that can be used to build novel functional devices, given the fact that bio-nanofibers can be decorated

by functional components such as magnetic, semiconducting, metallic, and bone mineral nanoparticles.^[22, 28]

Such long-range-ordered nanostructures may be used as unique scaffolds in tissue engineering for controlling the alignment and morphology of resident stem cells, which may in turn direct the proliferation and differentiation of stem cells due to the unique topography.^[27f] In addition, the bio-nanofibers we used are all genetically modifiable and can surface-display foreign peptides by genetic means,^[2a, 21b, 29] providing biochemical cues for directing stem cell behavior. For example, a RGD cell adhesion peptide^[30] can be genetically inserted into every subunit of the flagellum using our reported protocol,^[21b] allowing the full display of RGD peptide on the flagellum surface. Surface-displaying RGD peptide on bio-nanofibers can enhance cellular adhesion and growth on them.^[31] As a proof of concept, mesenchymal stem cells (MSCs) (1×10^4 cells/mL) were seeded onto the single-orientation parallel-aligned arrays (Fig. 1b) of MNP-decorated wild-type flagella (WT-flagellum-MNP) and MNP-decorated RGD-displayed flagella (RGD-flagellum-MNP) and their morphology was monitored. It was found that MSCs didn't adhere onto the WT-flagellum-MNP arrays (Figs. S16a). However, MSCs were found to adhere onto the RGD-flagellum-MNP arrays (Figs. S16b) due to the display of RGD cell adhesion peptide. More importantly, the MSCs adhered onto the RGD-flagellum-MNP arrays are elongated along the long axis of the flagella. Conversely, MSCs seeded on control cell culture plate adhered onto the plate but didn't show any morphological control (Fig. S16c) such as stem cell elongation, which was recently found to favour the stem cell differentiation^[32]. These results indicate that our long-range-ordered nanostructures (bio-nanofiber-MNPs) along with tunable surface chemistry (peptide display) are able to direct the fate of stem cells.

In summary, for the first time, we took advantage of the capability of MNPs being aligned along a magnetic field and reproducibly generate large scale bio-nanofiber assemblies with orientation of the constituent bio-nanofibers defined by the applied magnetic field. When decorated by MNPs, bio-nanofibers could be guided by the external magnetic field to become oriented horizontally (Figs. 1b&c) or vertically (Fig. 1d) with respect to the substrate surface, forming single- and multi-orientation assemblies. We believe that the magnetically directed assembly method is not limited to the three bio-nanofibers studied in this work, but can be extended to other filamentous supramolecular assemblies, for generating long-range-ordered nanostructures. Such long-range-ordered nanostructures can be used as magnetic scaffolds in tissue engineering for promoting the adhesion, proliferation, and differentiation of resident cells.^[33] In addition, since the bio-nanofibers, such as flagella, pili, and phage, can be bio-engineered to display functional peptides on their surfaces^[2a, 4b, 21b] and decorated with functional nanoparticles,^[22, 28] an oriented assembly of the peptide-displayed versions of these bio-nanofibers is expected to find potential applications in functional devices, materials science, magnetics, electronics, tissue engineering, and nanomedicine.

Experimental Section

Typical Alignment Experiments

Fe₃O₄ MNPs solution (typically 70 mg/ml, pH=4.0) was mixed with bio-nanofiber solution (flagellum solution, OD_{280nm}=0.1; pilus solution, OD_{280nm}=0.25; phage solution, 10¹³ pfu/mL; all bio-nanofibers dissolved in water with pH=7.0) and the mixture was allowed to incubate for 10 s at room temperature to form MNP-bio-nanofiber complexes, respectively, and then air-dried on a cover-slide in a constant magnetic field with a defined direction and strength (Fig. 1). Then the dried samples were directly visualized under a light microscope or coated with a layer of Pt and characterized by a SEM. The magnetic field used here was

generated within two parallel placed neodymium cylinder magnets. The magnetic lines in the magnetic field should be parallel with each other.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Jarrell KF, McBride MJ. *Nat Rev Microbiol.* 2008; 6:466–476. [PubMed: 18461074]
2. a) Cao BR, Xu H, Mao CB. *Angew Chem Int Edit.* 2011; 50:6264–6268. b) Hahn E, Wild P, Hermanns U, Sebbel P, Glockshuber R, Haner M, Taschner N, Burkhard P, Aebi U, Muller SA. *J Mol Biol.* 2002; 323:845–857. [PubMed: 12417198]
3. Kehoe JW, Kay BK. *Chem Rev.* 2005; 105:4056–4072. [PubMed: 16277371]
4. a) Wang FK, Cao BR, Mao CB. *Chem Mater.* 2010; 22:3630–3636. [PubMed: 20802794] b) Xu H, Cao BR, George A, Mao CB. *Biomacromolecules.* 2011; 12:2193–2199. [PubMed: 21520924]
5. Mao CB, Wang FK, Cao BR. *Angew Chem Int Edit.* 2012; 51:6411–6415.
6. Lee SW, Mao CB, Flynn CE, Belcher AM. *Science.* 2002; 296:892–895. [PubMed: 11988570]
7. Lin Y, Balizan E, Lee LA, Niu ZW, Wang Q. *Angew Chem Int Edit.* 2010; 49:868–872.
8. Iyyamperumal E, Wang SY, Dai LM. *Acs Nano.* 2012; 6:5259–5265. [PubMed: 22639830]
9. Cao AY, Zhang XF, Xu CL, Liang J, Wu DH, Chen XH, Wei BQ, Ajayan PM. *Appl Phys Lett.* 2001; 79:1252–1254.
10. Varghese OK, Paulose M, Grimes CA. *Nat Nanotechnol.* 2009; 4:592–597. [PubMed: 19734933]
11. Munshi AM, Dheeraj DL, Fauske VT, Kim DC, van Helvoort ATJ, Fimland BO, Weman H. *Nano Lett.* 2012; 12:4570–4576. [PubMed: 22889019]
12. Huang ZP, Shimizu T, Senz S, Zhang Z, Zhang XX, Lee W, Geyer N, Gosele U. *Nano Lett.* 2009; 9:2519–2525. [PubMed: 19480399]
13. Lai E, Kim W, Yang PD. *Nano Res.* 2008; 1:123–128.
14. Utama MIB, Peng ZP, Chen R, Peng B, Xu XL, Dong YJ, Wong LM, Wang SJ, Sun HD, Xiong QH. *Nano Lett.* 2011; 11:3051–3057. [PubMed: 21043505]
15. Frankel RB. *Annu Rev Biophys Bio.* 1984; 13:85–103.
16. Xie J, Chen K, Chen XY. *Nano Res.* 2009; 2:261–278. [PubMed: 20631916]
17. Ahniyaz A, Sakamoto Y, Bergstrom L. *P Natl Acad Sci USA.* 2007; 104:17570–17574.
18. a) Lynch MD, Patrick DL. *Nano Lett.* 2002; 2:1197–1201. b) Kumar S, Kaur H, Kaur H, Kaur I, Dharamvir K, Bharadwaj LM. *J Mater Sci.* 2012; 47:1489–1496.
19. Grigorova T, Pispas S, Hadjichristidis N, Thurn-Albrecht T. *Macromolecules.* 2005; 38:7430–7433.
20. Wang FK, Nimmo SL, Cao BR, Mao CB. *Chem Sci.* 2012; 3:2639–2645. [PubMed: 23630644]
21. a) Li D, Qu XW, Newton SMC, Klebba PE, Mao CB. *J Mater Chem.* 2012; 22:15702–15709. [PubMed: 22865955] b) Wang F, Li D, Newton SM, Klebba PE, Mao CB. *Adv Funct Mater.* 2009; 19:3355–3355.
22. Cao BR, Xu H, Mao CB. *Microsc Res Techniq.* 2011; 74:627–635.
23. Massart R. *IEEE T Magn.* 1981; 17:1247–1248.
24. Xia HB, Cheng DM, Xiao CY, Chan HSO. *J Mater Chem.* 2005; 15:4161–4166.
25. Reches M, Gazit E. *Nat Nanotechnol.* 2006; 1:195–200. [PubMed: 18654186]

26. Chen XL, Gerasopoulos K, Guo JC, Brown A, Wang CS, Ghodssi R, Culver JN. *Acs Nano*. 2010; 4:5366–5372. [PubMed: 20707328]
27. a) Gonzalez-Valls I, Lira-Cantu M. *Energ Environ Sci*. 2009; 2:19–34. b) Yu HY, Sun Y, Singh N, Lo GQ, Kwong DL. *Microelectron Reliab*. 2012; 52:651–661. c) Han WH, Zhou YS, Zhang Y, Chen CY, Lin L, Wang X, Wang SH, Wang ZL. *Acs Nano*. 2012; 6:3760–3766. [PubMed: 22537160] d) Hsu CL, Chen KC. *J Phys Chem C*. 2012; 116:9351–9355. e) Offermans P, Crego-Calama M, Brongersma SH. *Sensor Actuat B-Chem*. 2012; 161:1144–1149. f) Dalby MJ, Gadegaard N, Tare R, Andar A, Riehle MO, Herzyk P, Wilkinson CDW, Oreffo ROC. *Nat Mater*. 2007; 6:997–1003. [PubMed: 17891143] g) Oh S, Brammer KS, Li YSJ, Teng D, Engler AJ, Chien S, Jin S. *P Natl Acad Sci USA*. 2009; 106:2130–2135.
28. a) Cao B, Mao C. *Langmuir*. 2007; 23:10701–10705. [PubMed: 17850102] b) Mao CB, Flynn CE, Hayhurst A, Sweeney R, Qi JF, Georgiou G, Iverson B, Belcher AM. *P Natl Acad Sci USA*. 2003; 100:6946–6951. c) Mao CB, Solis DJ, Reiss BD, Kottmann ST, Sweeney RY, Hayhurst A, Georgiou G, Iverson B, Belcher AM. *Science*. 2004; 303:213–217. [PubMed: 14716009]
29. Zhu HB, Cao BR, Zhen ZP, Laxmi AA, Li D, Liu SR, Mao CB. *Biomaterials*. 2011; 32:4744–4752. [PubMed: 21507480]
30. Pierschbacher MD, Ruoslahti E. *Nature*. 1984; 309:30–33. [PubMed: 6325925]
31. a) Lee LA, Nguyen QL, Wu LY, Horyath G, Nelson RS, Wang Q. *Biomacromolecules*. 2012; 13:422–431. [PubMed: 22188342] b) Wang JL, Wang L, Li X, Mao CB. *Sci Rep-Uk*. 2013; 3. c) Rong JH, Lee LA, Li K, Harp B, Mello CM, Niu ZW, Wang Q. *Chem Commun*. 2008:5185–5187.
32. a) Subramony SD, Dargis BR, Castillo M, Azeloglu EU, Tracey MS, Su A, Lu HH. *Biomaterials*. 2013; 34:1942–1953. [PubMed: 23245926] b) Pignatello, R. *Advances in Biomaterials Science and Biomedical Applications*. Vol. 14. InTech; 2013.
33. a) Bock N, Riminucci A, Dionigi C, Russo A, Tampieri A, Landi E, Goranov VA, Marcacci M, Dediu V. *Acta Biomater*. 2010; 6:786–796. [PubMed: 19788946] b) Zeng XB, Hu H, Xie LQ, Lan F, Jiang W, Wu Y, Gu ZW. *Int J Nanomed*. 2012; 7:3365–3378.

Figure 1.

Schematic illustration of using magnetic field to guide the assembly of filamentous supra-biomolecular assemblies with the help of magnetic nanoparticles. (a) General idea of the method. Positively charged Fe_3O_4 MNPs interact with negatively charged bio-nanofibers to form MNP-bio-nanofiber complexes, which can be further guided by magnetic fields. (b) Single-orientation parallel-aligned bio-nanofiber arrays. (c) Multi-orientation multi-layered bio-nanofiber assemblies formed by twisting the direction of magnetic field for each neighboring layer. After one parallel aligned bio-nanofiber layer is formed under the guidance of one magnetic field (B1), another layer of parallel aligned bio-nanofiber layer is deposited under the guidance of a magnetic field with a different direction (B2). (d) Vertically aligned bio-nanofiber arrays guided by a magnetic field perpendicular to a substrate. (e), (f), and (g) Structures of three types of bacteria-related bio-nanofiber: bacterial flagellum (e) which protrudes from bacterial cell surface for assisting cell swimming, type 1 pilus (f) which is attached to the bacterial cell surface for assisting cell adhesion, and M13 phage (g) which is a virus specifically infecting bacteria.

Figure 2.

TEM images of Fe₃O₄ MNPs and MNP-bio-nanofiber complexes formed by the assembly of MNPs along different bio-nanofibers. (a) As-synthesized Fe₃O₄ MNPs. Inset: the synthesized Fe₃O₄ MNPs can be attracted by a magnet. (b) MNP-flagellum complexes. (c) MNP-pilus complexes. (d) MNP-phage complexes. In b–d, the nanoparticles are coated on the surface of the bio-nanofibers.

Figure 3. SEM images of the single-orientation horizontally aligned Fe_3O_4 MNPs and MNP-bio-nanofiber complexes in the presence of magnetic fields (B, indicated by arrows). (a) Aligned Fe_3O_4 MNPs. (b) Aligned MNP-flagellum complexes. (c) Aligned MNP-pilus complexes. (d) Aligned MNP-phage complexes. The corresponding optical images of MNP-bio-nanofiber complexes in the presence of magnetic fields can be found in supporting information (Fig. S6).

Figure 4.

Optical images of double-orientation multi-layered bio-nanofiber arrays. (a) Optical image of double-orientation layered MNP-flagellum complexes. (b) Optical image of double-orientation layered MNP-pilus complexes. (c) Optical image of double-orientation layered MNP-phage complexes. (d) The tilt view of the peripheral edge (intentionally broken to expose the two layers by removing some materials) of double-orientation layered MNP-pilus complex arrays. The similar view of double-orientation layered flagellum and phage arrays can be found in supporting information (Fig. S6).