Magnetically guided alignment of bio-nanofibers into ordered structures for controlling stem cell behaviors

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Statement of Purpose: Filamentous biomacromolecules, such as bacterial flagellum, [1] bacterial pilus, [2] and bacteriophage, [3] are a class of 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, these bio-nanofibers have been used as templates for directing the assembly of functional nanomaterials into ordered hierarchical structures for the fabrication of supramolecular structures and nanodevices. However, to induce their assembly normally requires control of concentration and the assembly in large scale is still challenging. Here we proposed to use magnetic field to guide the assembly of bio-nanofibers with the help of magnetic nanoparticles (Fig. 1). [4] The resultant assemblies were used to guide the stem cell growth and study the differentiation of stem cells.

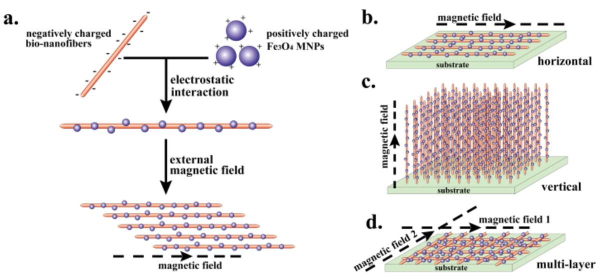

Figure 1. Schematic illustration.

Methods: To achieve horizontally parallel aligned bio-nanofiber arrays, 2 µl Fe_3O_4 MNPs solution (70 mg/ml) was mixed with 100 µL flagella solution (OD_{280nm}=0.1) or control solution (distilled water) completely and air-dried on a cover-slide in a constant magnetic field (1 T) whose direction was parallel to the cover-slide (Fig. 1b). For vertical alignment, 50 µL Fe_3O_4 MNPs solution (70 mg/ml) was mixed with 500 µL phage solution (5×10^{14} pfu/mL) and air-dried on a cover-slide in a constant magnetic field (1.5 T) whose direction was perpendicular to the cover-slide (Fig. 1c). In addition, after the complete drying of the first layer of horizontally aligned bio-nanofiber arrays, a mixed solution of Fe_3O_4 MNPs and nanofibers could be applied onto the first layer and air-dried in a rotated external magnetic field (Fig. 1d) to form double-layered bio-nanofiber assemblies.

Results: The SEM results (Fig. 2) showed that not only Fe_3O_4 MNPs (Fig. 2a) but also MNP-bio-nanofiber complexes (Figs. 2b&3) could be horizontally parallel aligned in the magnetic field perfectly. By simply changing the direction of the external magnetic field (Fig. 1c), vertically aligned phage arrays could also be achieved. The SEM result (Fig. 2c) showed that thousands of phage nanofibers were standing on the substrate side-by-side along the direction of magnetic field. Using a stronger magnetic field (1.5 T) and highly concentrated phage solution (5×10^{14} pfu/mL) are two critical factors to achieve such vertical alignment of nanofibers. We also tried to form the multi-layered bio-nanofiber assemblies. The SEM results (Fig. 2d) showed that the bio-nanofiber aggregates 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. By using this method, it is possible to construct long-range multi-layered bio-nanofiber structures with a controlled angle between two neighboring layers. In addition, we found the bio-nanofiber arrays formed in the presence of strong magnetic field (1 T) showed higher degrees of ordering than those formed in the presence of weak magnetic field (0.01 T) (Fig. 4). We found that once mesenchymal stem cells (MSCs) were cultured on the structures assembled from bio-nanofibers, they were aligned and elongated along the length direction of the bio-nanofibers (Fig. 5). We are now studying the osteogenic differentiation of MSCs on the structures shown in Fig. 2.

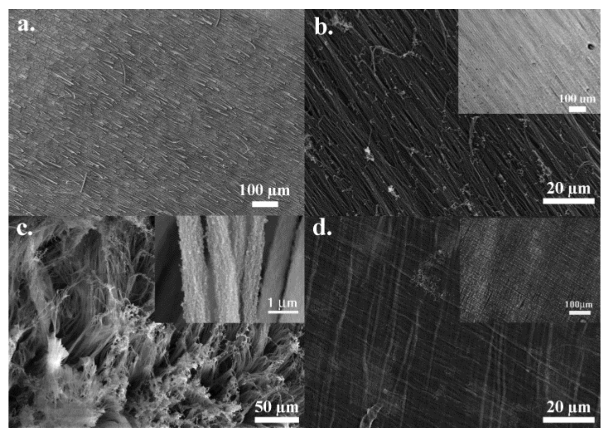

Figure 2. SEM results of magnetically oriented bio-nanofiber arrays.

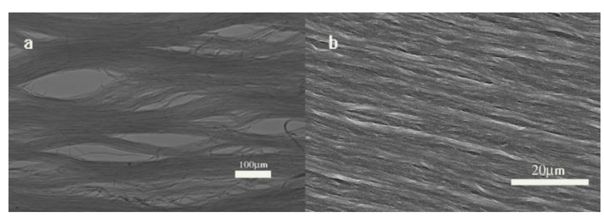

Figure 3. Magnetically oriented flagella arrays

Conclusions: In summary, for the first time, we took advantage of the alignment of MNPs in the presence of magnetic fields to reproducibly generate controlled long-range nanoarchitectures assembled from bio-nanofibers including phage, flagella and pili. When associated with MNPs, bio-nanofibers could be guided by the external magnetic field to form horizontally and vertically (Fig. 1) aligned single-layered bio-nanofiber arrays or multi-layered bio-nanofiber assemblies. Such long-range ordered nanofiber architectures can enable many possible applications in materials science, electronic device fabrication, tissue engineering, and nanomedicine.

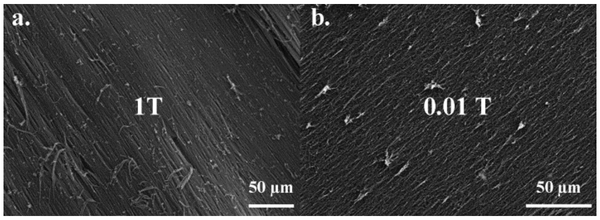

Figure 4. SEM results of magnetically oriented bio-nanofiber arrays with different strength of magnetic fields.

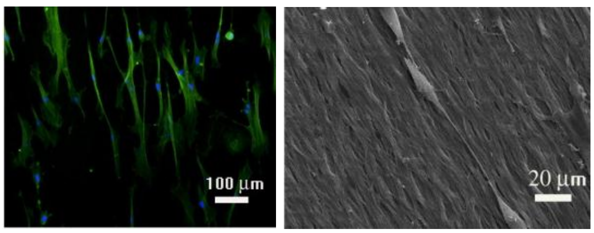

Figure 5. Typical morphologies of MSCs on aligned bionanofiber structures (left, fluorescence microscopy, right: SEM).

References:

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