

## Multiscale Organization of Nanofiber-based Structures: Nature Design, Bio-inspired Engineering and Future Directions

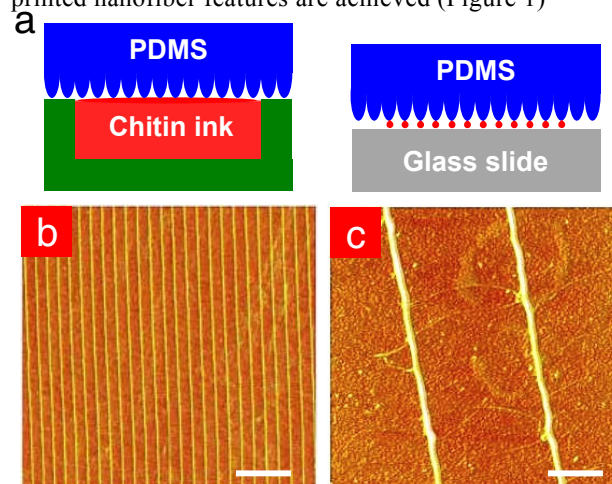
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**Introduction.** The ability to precisely manipulate, localize, and assemble biological and bioinspired molecules into organized structures has contributed to great advances in bionanotechnology including bioelectronics, biophotonics, tissue engineering, and regenerative medicine. Multi-scale organization of self-assembled nanofibers is particularly attractive because of their biocompatibility and self-assembly-enabled functionality. Nature has evolved with many strategies to weave well-ordered proteins and/or polysaccharide nanofibers into hierarchical structures or functional materials such as biophotonics, biocomposites and bacteria biofilms, and therefore provides inspiration for future design. Here, we introduce our recent efforts towards bio-inspired patterning of chitin nanofiber structures across multi-scale lengths based on a simple chitin nanofiber ink concept, with demonstrated application in biophotonics. In addition, we demonstrated that amyloid-based nanofibers can be genetically tuned for new functions without disrupting inherent self-assembly. Applications of patterned nanofiber structures in biophotonics, tissue engineering, and biocompatible devices can be envisioned. Finally, one unanswered but interesting question is how nature simultaneously directs spatial and temporal control of nanofiber patterning across multiscale dimensions. To answer that question will require deep understanding of biology, and in turn will promise new approaches towards bio-inspired patterning of nanofibers via synthetic biology.

**Materials & Methods** The chitin nanofiber ink was prepared following a previously published procedure.<sup>1</sup> PDMS stamps were fabricated using PDMS replica molding. Holographic optical diffraction gratings are used as masters. After inking, the PDMS was immediately placed in conformal contact with a glass slide. Molecular level tuning of amyloid fibers via gene engineering: DNAs encoding amyloid fiber subunits, CsgA and other functional domains were reconstructed into plasmids by Gibson assembly. Variants of CsgA protein was expressed in *E. coli* and purified using Ni/NTA column. Self-assembly of CsgA were carried out in PBS solution.

**Results & Discussion** Self-assembled chitin nanofibers are microcontact printed onto a glass substrate. Ultrafine nanofibers self-assemble from a hexafluoro-2-propanol

chitin solution upon drying. We use this “chitin nanofiber ink” concept for airbrushing, replica molding, and micro-contact printing of chitin nanofiber structures across length scales. Notably, sub-35 nm micro-contact printed nanofiber features are achieved (Figure 1)



**Figure 1.** (a) Microcontact printing of chitin nanofibers onto glass substrates. (b, c) Topographic AFM images of chitin nanofiber patterns printed from 0.05 (w/v)% ink. The PDMS stamp is replicated from a holographic diffraction grating with 1200 grooves/mm. Scale bar: (b) 4  $\mu\text{m}$ , (c) 400 nm

**Conclusion** Many hierarchical structures or functional materials in nature are woven by well-ordered proteins and/or polysaccharide nanofibers across multiscale dimensions and provide inspiration for new materials or structures design. Here, we showed that chitin nanofibers can be patterned across multiscale lengths coupling a bottom-up self-assembly with top-down soft lithography. We also demonstrated that amyloid fibers can be genetically tuned at molecular level for new functions without disrupting inherent self-assembly. Understanding how nature organizes nanofiber structures across multiscale dimensions will provide new insights towards bio-inspired patterning of nanofibers via synthetic biology.

### References

1. C. Zhong, *et al.*, *Soft Matter*, 2010, 6, 3908.
2. C. Zhong, *et al.*, *Advanced Materials*, 2011, 22, 6080.