

# 2D AND 3D FUNCTIONAL NANOSTRUCTURES OF GENETICALLY ENGINEERED SPIDER SILK USING FOCUSED ION BEAM

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## ABSTRACT

We report a new bionanomanufacturing technique for both 2d and 3d protein nanostructuring using focus ion beam (FIB) as the fabrication tool and genetically engineered recombinant spider silk as the resist material. Recombinant spider silk is mechanically strong, biocompatible, biodegradable and biofunctionalizable. Precise control of genetically engineered spider silk at the nanoscale offers new route to protein-based functional nanostructures.

## INTRODUCTION

Recent developments in nanotechnology have led to renewed interest and breakthroughs using biopolymers, specifically natural proteins, as novel functional materials [1–3]. Electron beam lithography (EBL) has been used intensively for precise nanopatterning. Recently, there has been important progress on “green” EBL using biomaterial-based photoresist such as naturally extracted silk fibroins [4–6]. In this work, we report a new processing technology for bionanomanufacturing using focused ion beam lithography (IBL) and genetically engineered recombinant spider silk as the resist material.

There are two major improvements in the work, compared to the traditional EBL technique. Firstly, IBL is generally faster than EBL and thus less time-consuming while offering high accuracy, mainly due to the higher

efficiency of the energy transfer of ions into solid materials than electrons. IBL is about 3 orders of magnitude faster than EBL for the same pattern manufacturing in our case. Secondly and more importantly, the expression of recombinant spider silk proteins can be controlled at the genetic level for desired performances and properties. For example, compared to silk fibroin proteins, recombinant spider silk proteins have better-controlled molecular weights. In our work, the molecular weight distribution for naturally extracted silk fibroin ranges from 30–250 kDa while genetically engineered spider silk proteins used in this work has been precisely controlled to be 72 kDa via genetic and protein engineering. Last but not least, generally spider silk has higher mechanical strength, elasticity, and toughness than silkworm silk. Therefore, we believe that IBL using recombinant spider silk will be a powerful tool for next-generation protein-based bionanomanufacturing.

## DESIGN AND FABRICATION

Similar as EBL for silk nanostructuring, FIB can be applied with well-controlled dosages and voltages to selectively crosslink or de-crosslink the recombinant spider silk and change its water-solubility, resulting in either negative nanostructures or positive nanostructures, respectively. Note that only water is used as the developing solution and no hazardous chemicals are used or generated.

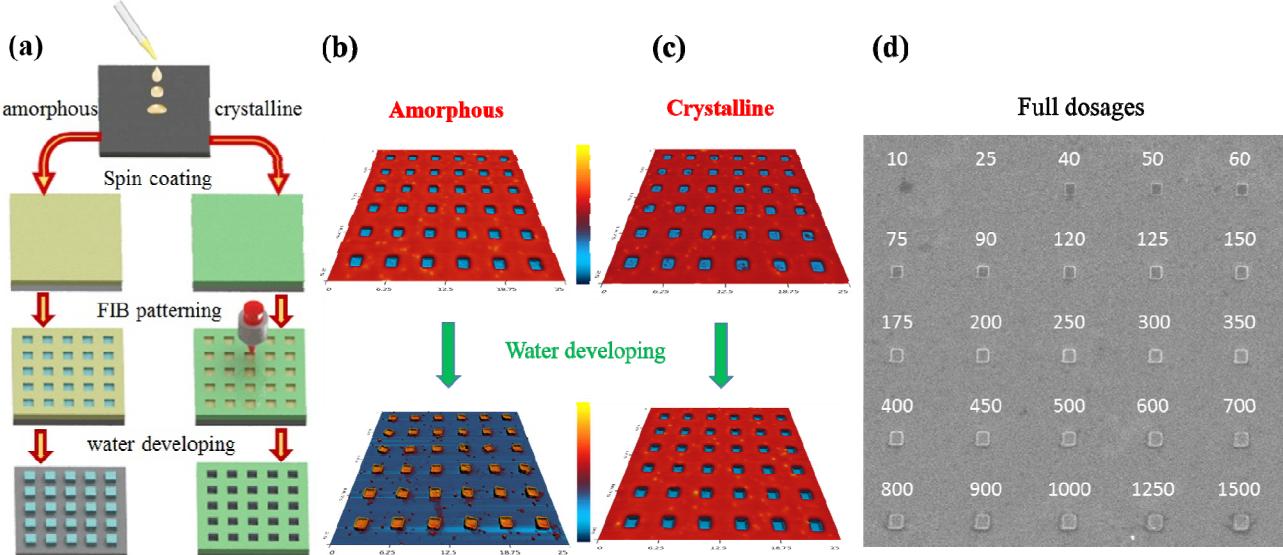


Figure 1: Schematic of ion beam nanolithography using recombinant spider silk as the resist material. (b) The AFM 3D images of as-fabricated nanopatterns before and after the water development fabricated on amorphous and crystalline spider silk by FIB. (c) The SEM image of nanopatterns fabricated by different ion beam dosages ranging from 10 to 1500  $\mu\text{C}/\text{cm}^2$ .

A typical process flow is illustrated in Fig. 1a. Thin spider silk films of about 200 nm were spin-coated on silicon substrates. For negative IBL, untreated amorphous spider silk film was used as a negative photoresist (Fig. 1b). For positive IBL, a pre-treatment (immersing the spider silk film in methanol for half a minute) was applied to crosslink the spider silk film which turned water-insoluble and therefore was used as a positive photoresist (Fig. 1c). The voltage and the ion beam current were precisely controlled so to expose the photoresist just enough to crosslink without etching it all off. The applied ion beam irradiated the silk film via the deposition of energy, causing structural changes in spider silk proteins (e.g., beta-sheets to random coils for their use in the positive tone). Water development was needed to dissolve and then washed away uncrosslinked or decomposed proteins and formed the defined nanopatterns/nanostructures.

## EXPERIMENTAL RESULTS

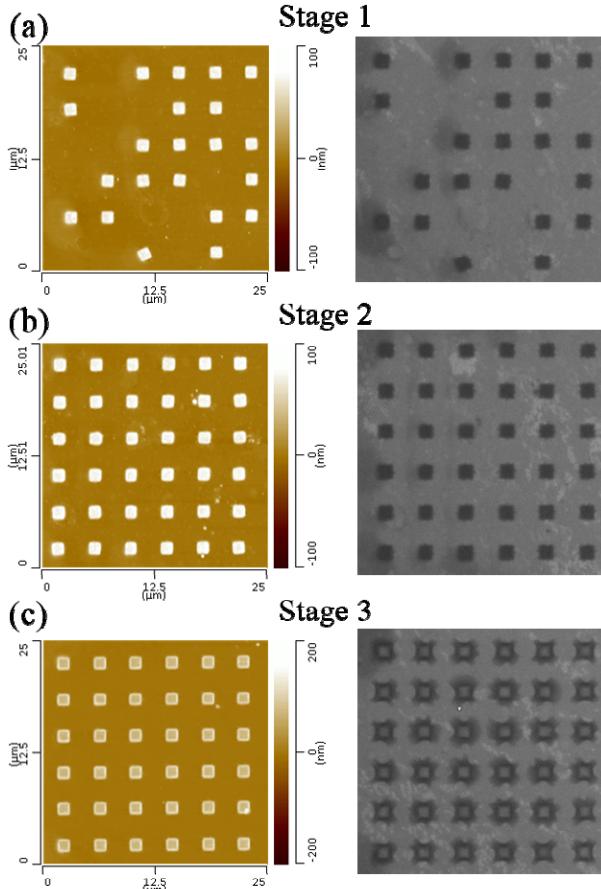


Figure 2. The AFM images (left column) and SEM images (right column) of three phase patterns at 40, 120, and 250  $\mu\text{C}/\text{cm}^2$ , separately.

When interacting with the spider silk, the ion beam can etch off part of the silk films (including both amorphous and crystalline ones) due to the high energy of as-applied ion beam. As shown in Fig. 1 (d) for negative nanostructuring, no square patterns were left on the substrate at small dosages (below 40  $\mu\text{C}/\text{cm}^2$  in our case). At the increased ion beam dosages, the patterns remained on the substrate. And at last, when the dosage is large

enough ( $> 250 \mu\text{C}/\text{cm}^2$ ), the silk proteins were overexposed and overetched, leading to a partial or total carbonization. This is quite different from the EBL. We carefully controlled the ion beam dosages to study the ion-protein interactions at the nanoscale. Three stages were found with each one corresponding to a different molecular structure during the nanofabrication of amorphous spider silk with increasing ion beam dosages.

As shown in Fig. 2, at small dosages (e.g., 40  $\mu\text{C}/\text{cm}^2$ , namely, stage 1), the applied ion beam was not able to fully crosslink the amorphous spider silk down to the bottom so that the partly crosslinked nanostructures rotated and translated from their original positions, as is shown in Fig. 2(a). When a proper dosage was applied (e.g. 120  $\mu\text{C}/\text{cm}^2$  in our case, namely, stage 2), the desired nanostructures were obtained, as shown in Fig. 2(b). At excessive dosages (e.g., 250  $\mu\text{C}/\text{cm}^2$ , namely, stage 3) overexposure and overetching occurred, resulting in rough edges and concave surfaces, as shown in Fig. 2(c).

IBL can fabricate varies of 2d patterns precisely, here we show a series of 2d nanopatterns that have been fabricated in both positive and negative tones, at resolutions down to  $\sim 50$  nanometers (Fig. 3a, 3b).

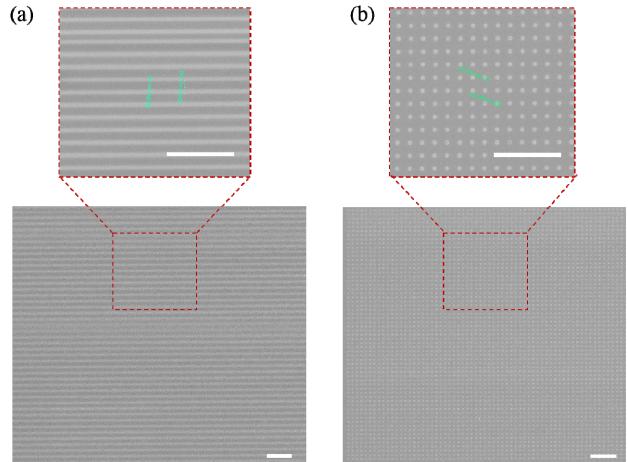


Figure 3. The SEM images of 2D nano gratings (a) and nano pillars (b) fabricated by IBL. The scale bar is 1  $\mu\text{m}$ .

More interestingly, we combined EBL (crosslinking occurring from bottom to top) and IBL (crosslinking occurring from top to bottom) together to manufacture a variety of complex 3d nanostructures which are difficult to make otherwise. For example, we patterned the periodic nano pillars on the substrate from the bottom using EBL. We then exposed the upper layer from the top using FIB. A water development was applied to obtain the 3D nanostructures (Fig.4a and 4b).

Furthermore, it is possible to develop biologically and optically functional bionanostructures by adding appropriate dopants (such as fluorescent dye molecules, quantum dots, collagen, growth factor, drugs and so on).

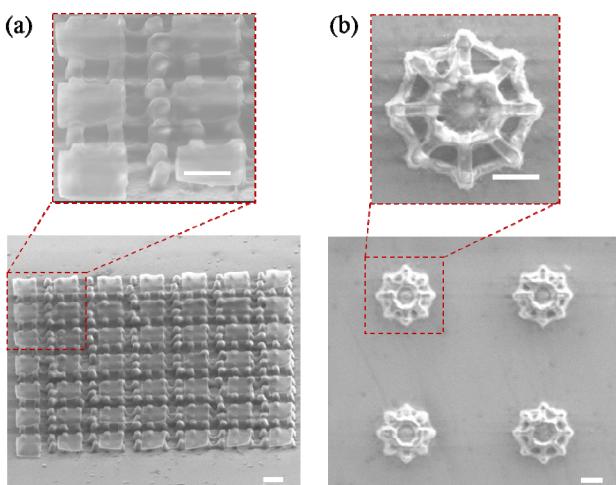


Figure 4. The SEM images of (a) 3d “nano-desks” and (b) 3d “nano-webs” fabricated by a combined nanolithographic process using IBL and EBL. The scale bar is 1  $\mu$ m.

## ACKNOWLEDGEMENTS

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