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Controlled assembly of multi-segment nanowires by histidine-tagged peptides

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Abstract
A facile technique was demonstrated for the controlled assembly and alignment of multi-segment nanowires using bioengineered polypeptides. An elastin-like-polypeptide (ELP)-based biopolymer consisting of a hexahistine cluster at each end (His_6-ELP-His_6) was generated and purified by taking advantage of the reversible phase transition property of ELP. The affinity between the His_6 domain of biopolymers and the nickel segment of multi-segment nickel/gold/nickel nanowires was exploited for the directed assembly of nanowires onto peptide-functionalized electrode surfaces. The presence of the ferromagnetic nickel segments on the nanowires allowed the control of directionality by an external magnetic field. Using this method, the directed assembly and positioning of multi-segment nanowires across two microfabricated nickel electrodes in a controlled manner was accomplished with the expected ohmic contact.

1. Introduction

One-dimensional (1D) nanostructures such as nanowires (NWs), nanotubes (NTs), nanospring and nanorods provide exciting building blocks for nanoscale electronic devices and sensors, because of their unique electronic and optical properties and the available synthesis methods for controlled shapes and sizes [1, 2]. Successful implementation of these nanostructured materials into functional devices requires the ability to spatially control and address these nanomaterials into a defined pattern [3, 4]. Several physical or chemical methods, such as post-growth assembly [5], magnetic entrapment and electric-field assisted alignment [6, 7], the Langmuir–Blodgett method, and microfluidic techniques [8], have been used, with mixed success. Biological assembly of nanostructures into functional devices has attracted increasing interest because of the highly specific nature offered by biomolecules such as proteins and DNA. Conjugation of biomolecules onto nanostructures can be used to tether the growth of extended nano-networks in a spatially controlled manner. For example, interactions based on receptor–ligand pairs such as streptavidin/biotin and antibody/antigen have been exploited as specific recognition for the multi-directional growth and positioning of nanostructures [9–11].

Besides receptor–ligand interactions, direct interaction between peptides and inorganic materials has also been demonstrated [12]. One example is the use of poly-histidine tags (his-tag), which are known to interact with Ni^{2+} and have been used for affinity purification of his-tagged proteins [13, 14]. Recently, it has been demonstrated that his-tagged proteins can also bind tightly onto three-segment Au/Ni/Au nanorods, presumably due to the presence of Ni^{2+} ions by surface oxidation, and can be separated by an applied magnetic field [15]. In this paper, we demonstrate the ability to selectively functionalize the nickel segments in a multi-segment nanowire (NW) with peptides containing a his-tag at each end, enabling the self-assembly of nanowires and the directed positioning of nanowires onto nickel surfaces (figure 1). The presence of the ferromagnetic nickel segments allows control of the spatial orientation of the nanowires in the presence of an external magnetic field. Using this method, three-segment Ni/Au/Ni nanowires were synthesized and anchored onto the surface across two microfabricated nickel electrodes in a controlled manner to demonstrate the fabrication of nano-interconnects.

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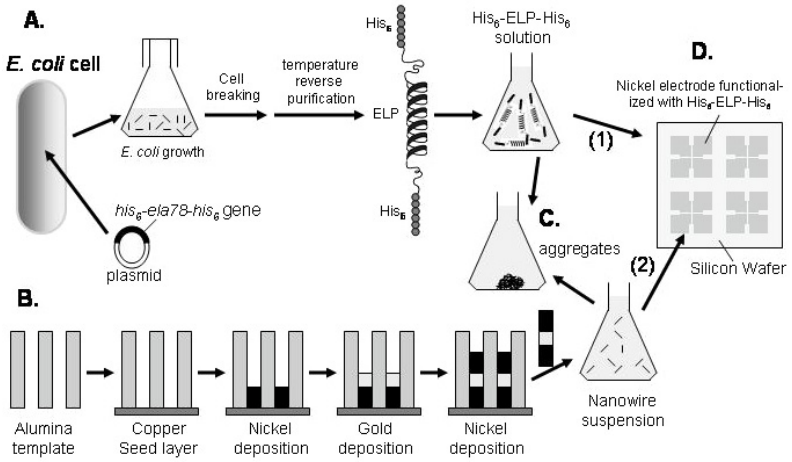

Figure 1. A schematic diagram depicting the strategies applied in this paper: (A) generation and purification of biopolymer His_6-ELP-His_6; (B) Ni/Au/Ni nanowire production; (C) aggregation of Ni/Au/Ni nanowires upon interaction with biopolymers; (D) deposition of Ni/Au/Ni nanowires onto nickel electrodes by biopolymers.

2. Experimental details

2.1. Construction of H6Ela38H6 and H6Ela78H6 expressing vectors

Vectors pELA38H6 and pELA78H6 were digested with *Nde*I and *Bam*HI and the excised fragment coding for the elastin genes with 38 (78) VPGVG repeats containing a C-terminal histidine tag were cloned into a T7-based expression vector pET14b+ possessing a N-terminal histidine-tag (from Novagen, Madison, WI, USA) digested the same way. The resulting vectors pH6Ela38H6 and pH6Ela78H6 were transformed into a production strain *E. coli* BLR(DE3) (Novagen).

2.2. Production and purification of biopolymer His_6-ELP-His_6

A single colony of *E. coli* cells (strain BL21(DE3), harbouring plasmid pHisEla78His) from a freshly revived culture on an LB/agar medium plate was inoculated into 5 ml of LB medium containing 100 μg ml^{-1} of ampicillin and grown overnight at 37 °C. The inoculum was transferred into a 250 ml TB broth containing 100 μg ml^{-1} of ampicillin and grown at 30 °C for 48 h with shaking at 300 rpm.

Cells were harvested by centrifugation at 3000 rpm for 15 min at 4 °C. The supernatant was decanted and the pellet was washed twice with 50 ml of 0.9% NaCl and resuspended in 25 ml of 50 mM Tris-Cl pH 7.4. Lysozyme (10 mg ml^{-1}) was added into the cell suspension to a final concentration of 1 mg ml^{-1} and kept on ice for 15 min. The suspension was then sonicated for 15 min, following a program of 5 s impulse/5 s pause, for cell lysis. The cell lysate was centrifuged at 15 000 rpm for 15 min at 4 °C. The supernatant was carefully collected and its volume was measured, and the pellet was discarded.

Based on the volume of the cell lysate, 5 N NaCl was added up to a final concentration of 1 N. The lysate was heated to 37 °C and maintained for 15 min in a water bath, during which the His_6-ELP-His_6 precipitation can be visualized as the solution became turbid. The precipitated proteins were recovered as a pellet by centrifugation at 37 °C for 15 min at 15 000 rpm.

To remove contaminating proteins co-precipitated with His_6-ELP-His_6, a second cycle of purification was performed. The recovered pellet was resuspended in 10 ml of ice-cold 50 mM Tris-Cl buffer pH 7.4 and centrifuged at 4 °C for 15 min at 15 000 rpm. The remaining pellet containing contaminating proteins was discarded and the supernatant was saved. This procedure was repeated once more before the supernatant was resolublized in 10 ml of ice-cold 50 mM Tris-Cl pH 7.4.

The purity of the purified ELP protein was investigated by analysing the samples from each step of the purification procedure by SDS-PAGE gel. Protein bands were visualized using the BioRad silver staining kit.

2.3. Synthesis of multi-segment Ni/Au/Ni nanowires

Multi-segment nanowires were synthesized using template-directed electrochemical deposition. Anodized alumina template (Anodisc 13 from Waterman Inc., with a nominal pore diameter of 200 nm) was used as a scaffold, with a metallic layer on one side serving a seed layer. A copper seed layer with a thickness of about 500 nm was sputtered using an Emitech K550 table-top sputter. The template was fixed to a glass support with the seed layer face down using double-sided conductive copper tape. Single-sided conductive copper tape was used as a lead to the glass support. The entire sample, except for the middle of the template and the end of the copper tape lead, was masked with insulator (Microstop, from Pyramid Plastics Inc.).

The nanowires were deposited in a constant-current mode with the template serving as the cathode. Soluble nickel anodes were used in nickel electrodeposition, and platinum-coated titanium anodes were used as the inert electrodes for gold electrodeposition. The nickel segments were electrodeposited from nickel sulfamate bath with boric acid as a pH buffer. The pH of the solution was adjusted to 4 by adding H_2SO_4 or NaOH. Ni electrodeposition was conducted at 10 mA cm^{-2} at ambient conditions without agitation. The gold segments

were electrodeposited from a ready-to-use cyanide-free gold bath (Technic RTU-25) at 2 mA cm^{-2} at ambient conditions to prevent discontinuous interfaces between gold segment and nickel segments, and agitated by a 1 inch stir bar in a 150 ml beaker at 100 rpm. All materials were electrodeposited using a multichannel EG&G PAR VMP2 potentio/galvanostat. The segmented nanowires were constructed by depositing Ni, changing the electrolyte for the gold mid-section, and switching back to a nickel bath. The lengths of different segments were controlled by adjusting the deposition time at a given current density. The template electrodes were rinsed with Nanopure water between plating solutions to prevent cross-contamination.

The nanowire-embedded template was removed from the substrate by detaching the copper tape lead and sonicating the glass support in acetone until the template was detached from the support. The alumina template was sonicated again in clean acetone and then isopropyl alcohol for another 1 min to remove the copper tape conductive adhesive residue from the backside of the template. The seed layer was removed from the template using copper etchant (ferric chloride). The template was dissolved in 1 M NaOH solution for 8 h at 60 °C. The nanowires were washed with water three times and the solution phase was discarded. Finally, the nanowires were suspended in 1 ml of Nanopure water and sonicated.

2.4. Biopolymer-facilitated aggregation of Ni/Au/Ni nanowire

A nanowire working suspension of 1 ml was first shaken well to disperse the nanowires, and a sample of 2 μl was taken out as a control sample. 100 μl of His_6-ELP-His_6 peptide solution was added into the well-dispersed nanowire suspension, mixed well and incubated for 5 min on a Labquake® Shaker Rotisserie (from Barnstead International, Dubuque, Iowa, USA), with periodic observation. Finally, an aliquot of 10 μl of the wire suspension was deposited onto a gold wafer surface, together with the control sample, for both optical microscopy (OM) and scanning electronic microscopy (SEM). As controls, ELP peptide with no his-tag and ELP peptide with his-tag on one end were used to incubate with the nanowire suspension, and observed with OM and SEM as well.

2.5. Enumeration of Ni/Au/Ni nanowires in the suspension

A working suspension of Ni/Au/Ni nanowires was diluted in a 10-fold series. 1 μl of a 10 000-fold diluted suspension was deposited onto a glass slide, dried and observed under the optical microscope. The number of individual nanowires was counted under the microscope using a haemocytological counter for different samples.

2.6. Biopolymer-facilitated positioning of Ni/Au/Ni nanowires

A silicon wafer with patterned nickel electrodes was first cleaned with 0.3 M sulfuric acid, cleansed with nanopure water and dried. The wafer was then submerged into 1 ml of His_6-ELP-His_6 peptide solution (diluted 1:10 from stored aliquot) and incubated for 5 min with mild agitation on a Titer Plate shaker (Lab-line Instruments, Inc., Melrose Park, IL, USA). The wafer was then removed, washed with Tris buffer (50 mM Tris buffer pH 7.4) three times and sonicated for 5 min at medium power during the second washing. Another wafer without modification by peptide solution was used as a control. To align the nanowires magnetically, the wafer was placed between two small ceramic magnets for positioning. The magnetic field strength was fixed at 200 G.

For site-specific positioning of nanowires, 1 μl of a 10 000-fold diluted nanowire suspension was added onto the wafer. The electrode surfaces were observed under an optical microscope and the number of nanowires was counted and recorded. The wafers were then washed with Tris buffer three times and sonicated for 5 min at medium power during the second washing. The two wafers were then dried, the surface of the nickel electrodes was observed under the optical microscope, and the number of nanowires on the nickel surface was counted.

2.7. Site-specific positioning of nanowires using biopolymer

An aliquot of 0.1 μl His_6-ELP-His_6 peptide solution was added onto a defined region of the nickel electrode surface using a microsyringe. This drop covered part of the base electrode, part of the microelectrode tip, the region of silicon surface surrounding the microelectrode tip, and the gap between the two electrodes. After brief rinsing with nanopure water, the wafer was covered with a layer of well-dispersed nanowires suspension and incubated for 5 min with mild agitation on a Titer Plate shaker. The wafer was then washed with Tris buffer three times and sonicated for 5 min at medium power during the second washing. After the wafer was dried, the defined region modified with peptide solution was observed under the optical microscope.

3. Results and discussion

Segmented Ni/Au/Ni nanowires were fabricated by template-directed electrochemical deposition using commercially available anodized alumina (with an average pore diameter of 200 nm) as the nanotemplate (figure 1(B)). A gold mid-section was chosen for the initial proof-of-concept since gold is not known to interact with the his-tag. However, this segment can be easily interchangeable with a variety of materials amenable to electrodeposition, such as metals, metal oxides, semiconductors, and conducting polymers. The resulting nanowires were 200 nm in diameter with 3 and 8 μm long nickel segments and a 5 μm long gold middle segment (figure 2).

A synthetic gene coding for a peptide containing a hexahistidine tag at both ends was constructed. An elastin-like peptide containing 78 repeating GVGVP units was inserted between the two his-tags to allow easy purification of the His_6-ELP-His_6 peptide by inverse temperature cycling (figure 1(A)). Upon the addition of a His_6-ELP-His_6 peptide solution to a well-dispersed Ni/Au/Ni nanowire suspension (figure 2(A)), aggregation of the nanowires became immediately visible. An aliquot of the nanowire suspension was deposited onto a silicon wafer and dried before imaging. SEM images of the assembled structures revealed the presence of highly bundled structures (figure 2(B)) as well as some end-to-side (figure 2(B)) and end-to-end connections (figure 2(C)). To visualize the direct interaction between the His_6-ELP-His_6 peptide and the nickel segment, high-resolution optical microscopy was used. As can

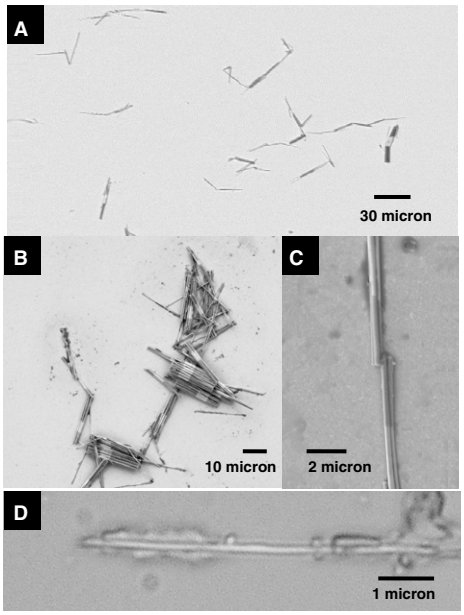

Figure 2. SEM images showing the dispersed Ni/Au/Ni nanowires (A) and the assembled nanostructures of nanowires by His_6-ELP-His_6 biopolymers ((B) and (C)), and an optical image demonstrating the specific interaction between the Ni segments and the His_6-ELP-His_6 biopolymers (D).

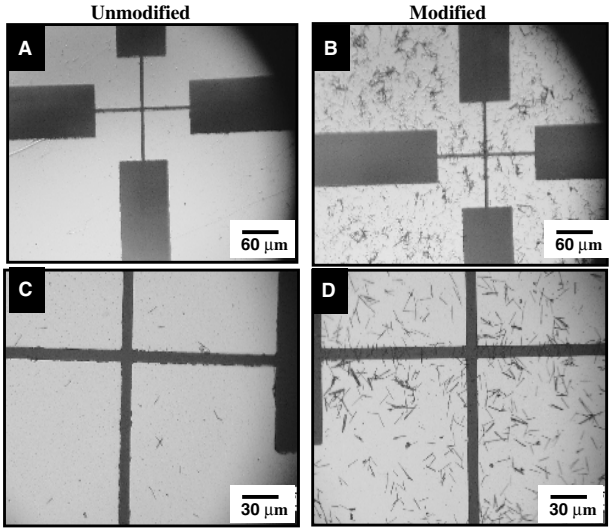

Figure 3. Optical micrographs of Ni/Au/Ni nanowires deposition onto unmodified ((A) and (C)) and modified ((B) and (D)) nickel electrodes.

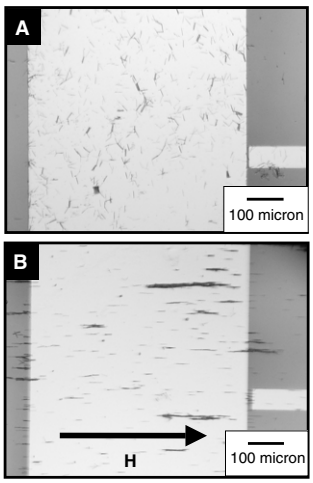

Figure 4. Optical micrographs of Ni/Au/Ni nanowires deposition onto peptide-functionalized electrode surfaces in the absence (A) or presence (B) of an external magnetic field in-plane to the electrodes (indicated by an arrow).

be seen in figure 2(D), only the nickel segments were shown to interact with the peptides. To verify that the linkages between the NWs were due to interaction between the nickel segments and the His_6-ELP-His_6 peptides rather than overlapping of NWs, ELP peptides without any his-tag or with only one his-tag were used with no noticeable assembled structures detected (data not shown).

To demonstrate the positioning of nanowires onto nickel surfaces using the His_6-ELP-His_6 peptide, a patterned nickel electrode was first modified with His_6-ELP-His_6 peptides by adding 1 μl of protein solution. After washing the electrode three times with 50 mM Tris buffer pH 7.4 and sonicating the electrodes with medium power during the second washing in the buffer for 5 min, a 1 μl suspension of Ni/Au/Ni nanowires was added on top of the electrode. After 30 min of incubation, the suspension was removed, washed three times with Tris buffer, and sonicated for 5 min with medium power to remove any non-specific interaction with the surrounding silicon surfaces. As a control, a nickel electrode without modification with the His_6-ELP-His_6 peptide was also used for comparison. As depicted in figures 3(B) and (D), most Ni/Au/Ni nanowires were deposited onto the nickel electrode surface (lighter region), while only a few were found on the silicon dioxide surface (darker region). More importantly, most deposited nanowires on the electrode surface existed as individual nanowires, with very few found as bundled nanowires. Interaction between the his-tag and the nickel materials appears to be strong, as the nanowires remained bound on the surface even after sonication (figure 4). In contrast, for the unmodified electrode, only a small number of nanowires remained bound to the surface after washing (figures 3(A) and (C)). Most importantly, only the modified nickel surfaces promoted the bridging of nanowires across the 5 μm gap between two adjacent electrodes (figure 3(D)). Since minimal background binding to the silicon dioxide surface or the unmodified nickel surface was observed, this result suggests that the His_6-ELP-His_6 peptides are solely responsible for the selective positioning of the segmented nanowires across the nickel electrodes. The presence of the nickel segments allows the guided orientation of nanowires with an external magnetic field. For example, when a small external magnetic field (≈200 Oe) was applied in-plane to the electrode surface, the tethered nanowires rotated so that the nickel segments were magnetically aligned to the field as shown in figure 4.

To demonstrate the possibility of site-specific positioning of nanowires on a defined region of the electrode surface, a small drop (0.1 μl) of His_6-ELP-His_6 solution was added onto the electrode surface. After incubation with nanowires and washing, the positioning of nanowires was observed using a

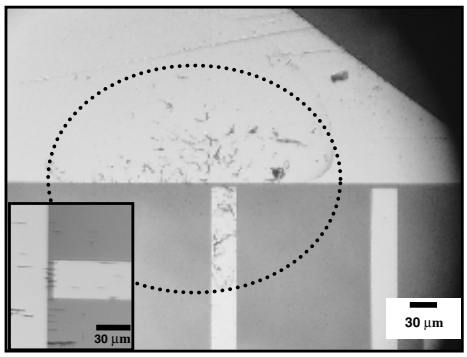

Figure 5. An optical micrograph of the site-specific deposition of nanowires. Area exposed to 0.1 μl of His_6-ELP-His_6 solution was highlighted by a circle. Bridging of electrodes by nanowires using an applied magnetic field is shown in the inset.

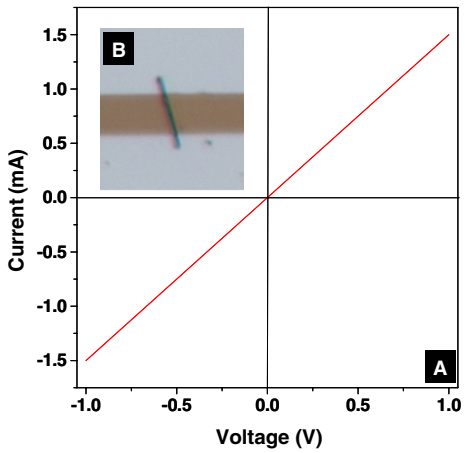

Figure 6. A current versus voltage (I–V) curve of a single Ni/Au/Ni nanowire bridging two nickel electrodes at a 50 mV s^{-1} scan rate. The inset is an optical image of the bridging Ni/Au/Ni nanowire.

(This figure is in colour only in the electronic version)

high-resolution optical microscope. Nanowires were detected only in the area exposed to the His_6-ELP-His_6 peptides (highlighted by the circle), while the unmodified area was free of nanowires (figure 5). The randomly distributed nanowires were aligned across the electrodes by applying an external magnetic field to provide the appropriate orientation (figure 5 inset). When a 10 000-fold dilution of the nanowire suspension was applied to the electrode surface, only a single nanowire was found to bridge the 5 μm gap between two adjacent nickel electrodes (figure 6(B)). To investigate whether this individual nanowire was functionally connected between the electrodes, an I–V characteristic was performed (figure 6(A)). The resistance for the nanowire was 1.51 kΩ or a resistivity of 9.44×10^{-6} Ω m. This is two orders of magnitude greater than the resistivity of bulk gold and may be attributed to the less conductive protein layer sandwiched between the nickel electrodes and the nanowire.

4. Conclusion

In summary, we report a facile technique for the controlled assembly and positioning of multisegment Ni/Au/Ni nanowires using polypeptides containing a his-tag at each end. The specific interaction between the his-tag and the nickel segment allows the rapid and stable assembly of nanostructures and the guided assembly of nanowires across pre-patterned electrodes. The presence of the ferromagnetic nickel segments on the nanowires also enables the control of spatial orientation by an external magnetic field. The ability to incorporate a wide range of materials as the mid-segment provides the feasibility to fabricate different functional devices using the same reported platform.

Acknowledgments

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