



Stretchable Electrochemical Sensor for Real-Time Monitoring of Cells and Tissues

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Abstract: Stretchable electrochemical sensors are conceivably a powerful technique that provides important chemical information to unravel elastic and curvilinear living body. However, no breakthrough was made in stretchable electrochemical device for biological detection. Herein, we synthesized Au nanotubes (NTs) with large aspect ratio to construct an effective stretchable electrochemical sensor. Interlacing network of Au NTs endows the sensor with desirable stability against mechanical deformation, and Au nanostructure provides excellent electrochemical performance and biocompatibility. This allows for the first time, real-time electrochemical monitoring of mechanically sensitive cells on the sensor both in their stretching-free and stretching states as well as sensing of the inner lining of blood vessels. The results demonstrate the great potential of this sensor in electrochemical detection of living body, opening a new window for stretchable electrochemical sensor in biological exploration.

Stretchable sensors have tremendous future applications in many fields, especially for health monitoring owing to closeness to the soft, elastic and curved properties of human body.^[1] Thanks to the development of novel nanomaterials and processing techniques in electronics,^[2] stretchable sensors have achieved significant progress in health-related physical sensing.^[1a,3] For the knowledge of chemical biomolecules regulating the sophisticated functions of biology, an electrochemical sensor is recognized to be a forceful tool capable of providing important chemical information with fast response and excellent sensitivity.^[4] In vivo, a large number of chemical molecules are highly related to mechanical deformation of cells and tissues. Exemplarily, mechanotransduction is an essential process by which cells convert mechanical stimuli into biochemical responses.^[5] Recently, initial studies have shown the unique advantage of an electrochemical sensor in detecting mechanically induced chemical release from

stretched cells.^[6] Further considering the deformation of cells and tissues during mechanotransduction, electrochemical sensor with favorable stretchability would expect to be an ideal tool to conform with cells and tissues deformation for real-time monitoring these chemical signals. However, despite significant achievements in electrochemical devices with substrate-skin elasticity,^[7] no impressive progress was witnessed in electrochemical sensors with excellent electrochemical performance and large strain tolerance for biological detection.^[8]

To fabricate stretchable electrochemical sensor, the conductive materials should satisfy both competent electronical conductivity and electrochemical activity. Among the most attractive nanomaterials [carbon nanotubes (CNTs), graphene, and metal nanowires (NWs)] currently used for stretchable electronics,^[2,9] randomly distributed metal NWs networks have received much attention because of their excellent mechanical stretchability and higher conductivity, compared with CNT networks and graphene films.^[2d,e] Ag NWs is the most extensively studied metal NWs for its superior electrical conductivity^[10] and convenient synthesis.^[11] Nevertheless, Ag NWs networks failed to serve as a working electrode firsthand because of the lack of electrochemical inertness in electrolyte solution. It is conceivable that Au NWs or NTs should be an ideal material to construct stretchable electrochemical sensor in view of the excellent performance of Au in electrochemical sensing.^[12] However, it is still a great challenge to routinely fabricate 1D Au NWs or NTs with a large aspect ratio.^[13] This explains why no work has been undertaken so far on Au NW- or NT-based stretchable electronics and sensors.

Here, we synthesized Au NT networks using mild galvanic displacement of sacrificial Ag NWs on polydimethylsiloxane (PDMS) film, and developed a stretchable electrochemical sensor for real-time monitoring of cells and tissues. As-prepared Au NTs/PDMS electrode demonstrates satisfying stability against mechanical deformation and excellent electrochemical performance. By culturing cells on this sensor, real-time monitoring of nitric oxide (NO) release from mechanically sensitive human umbilical vein endothelial cells (HUVECs) in both their stretching-free and stretching states was achieved. Furthermore, we rolled up this sensor to interface with the circular lining of elastic human vein and successfully monitored NO release therein. This work represents a first step toward the application of stretchable electrochemical sensors at the cell and tissue levels.

The stretchable Au NTs/PDMS film was fabricated using in situ galvanic displacement of sacrificial Ag NWs (10–20 μm in length) that had been pre-distributed uniformly on

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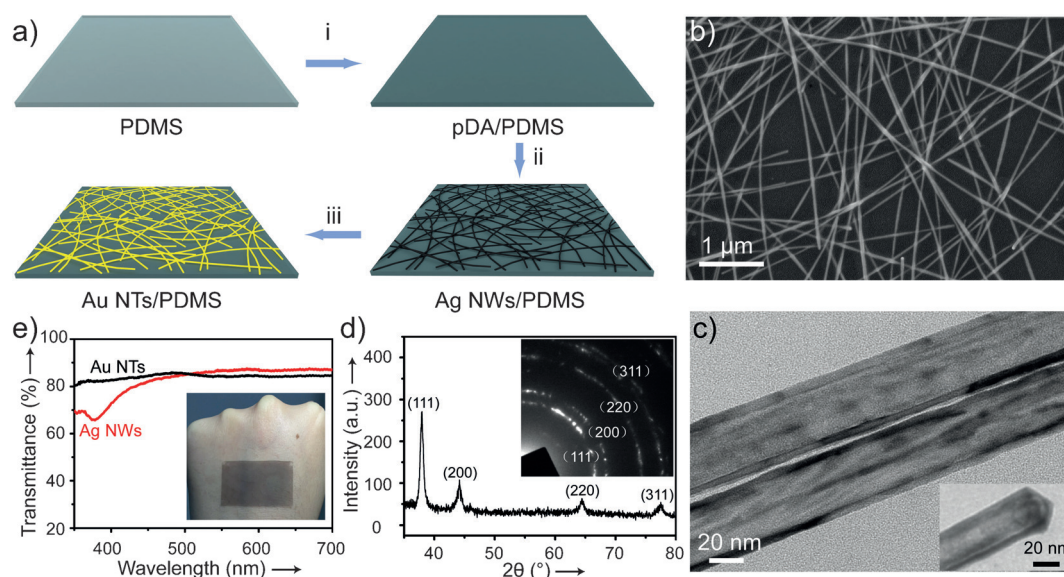
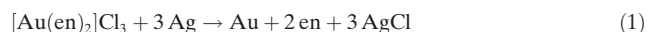


Figure 1. a) Fabrication of stretchable Au NTs/PDMS films. i) Treatment of the PDMS surface with pDA. ii) Spin-coating Ag NWs on PDMS. iii) Galvanic replacement of Ag NWs to Au NTs. b) SEM image of Au NTs/PDMS. c) TEM image of Au NTs. The inset shows the top end of an Au NT. d) XRD pattern of Au NTs. The inset is the SAED pattern of Au NTs. e) Optical transmittance of transparent Ag NWs (spin-coated with 2 mg mL^{-1} solution for 1 time) and resultant Au NTs on PDMS. The inset shows a photograph of Au NTs/PDMS film on the back of a hand.

poly-dopamine (pDA) treated PDMS (Figure 1a). The pDA layer endowed hydrophobic PDMS with a highly hydrophilic surface to enhance the adhesion between Ag NWs and the PDMS surface (see Figure S1 in the Supporting Information),^[14] and uniform Ag NWs networks were deposited on pDA-functionalized PDMS by controllable spin-coating (Figure S2). Au NTs/PDMS film was formed through the mild galvanic replacement between as-prepared Ag NWs networks and ethylene diamine (en) complex of gold, $[\text{Au}(\text{en})_2]\text{Cl}_3$ solution [Eq. (1)]. After exchange reaction, the byproduct solid AgCl was eliminated by a complexation reaction [Eq. (2)].



The elemental proportion of Au in Au NTs increased as the galvanic replacement advanced (Figure S3 and Table S1), while over-reaction leads to porosity and pinholes in the wall of Au NTs (Figure S4). Considering the balance of electrochemical behavior and sheet resistance, 60 minutes were chosen as the optimal reaction time at 90°C in 1 mm $[\text{Au}(\text{en})_2]\text{Cl}_3$ solution. Au elemental mass fraction of Au NTs is $91.2 \pm 2.1\%$ (mean standard deviation, $\pm \text{sd}$, $n=3$), indicating that almost all of Ag atoms have been replaced by Au. Scanning electron microscope (SEM) showed that the resultant Au nanomaterial distributed randomly on PDMS and perfectly inherited the wire-like morphology of Ag NWs templates (Figure 1b and Figure S1c) with large length-to-diameter ratio, which was beneficial to construct stretchable electrodes. Transmission electron microscopy (TEM) image (Figure 1c) revealed the hollow tubular structure of the obtained Au with a uniform outer diameter of about 40 nm

and wall thickness of about 4 nm. X-ray diffraction (XRD) peaks and the selected area electron diffraction (SAED) pattern (Figure 1d) indicated the (111), (200), (220), and (311) crystal planes of Au NTs, respectively.

Transmittance spectra of the initial Ag NWs and resultant Au NTs networks on PDMS were measured in the visible region of 350–700 nm (Figure 1e). The respective transmittance at 550 nm are 85.4% and 81.7%, and the corresponding sheet resistance are $18.9 \pm 0.9 \text{ } \Omega/\text{sq}$ and $23.1 \pm 1.1 \text{ } \Omega/\text{sq}$ (mean $\pm \text{sd}$, $n=5$), demonstrating these Au NTs have nearly maintained the excellent optoelectronic properties of Ag NWs (Figure S5). Besides, the Au NTs/PDMS film presented here is surface-compliant (Figure 1e, inset), and patterns and arrays of Au NTs/PDMS can be easily obtained (Figure S6). Cyclic voltammetric (CV) results with a pair of well-defined, reversible redox peaks at 0.17 V and 0.22 V in $\text{K}_3[\text{Fe}(\text{CN})_6]$ (Figure S3c,d), showed the excellent ability of Au NTs/PDMS in electron transport and electrochemical sensing.

Au NTs/PDMS film was connected with a light-emitting diode (LED) to investigate the stability of electrical conductivity. The LED remained lit when Au NTs/PDMS film was stretched to a strain of 50% (Figure S7), indicating the electron pathways through Au NTs networks are continuous in the strain range of 0–50%. To test the flexibility, Au NTs/PDMS film was wrapped on cylindrical objects with different curvatures and folded in half (Figure 2a). No significant change in the relative resistance ($\Delta R/R_0$) occurs for the bending to radii of curvature as small as 0.5 mm. As for stretchability (Figure 2b), $\Delta R/R_0$ reached 47% at a maximum strain of 50% and recovered partially with a final increase of 18% after full release in the first cycle. This can be ascribed to that the randomly distributed NTs can rotate and slide against each other,^[2d] while small portion of Au NTs may fail to interlace with others under large strain (Figure S8a). When

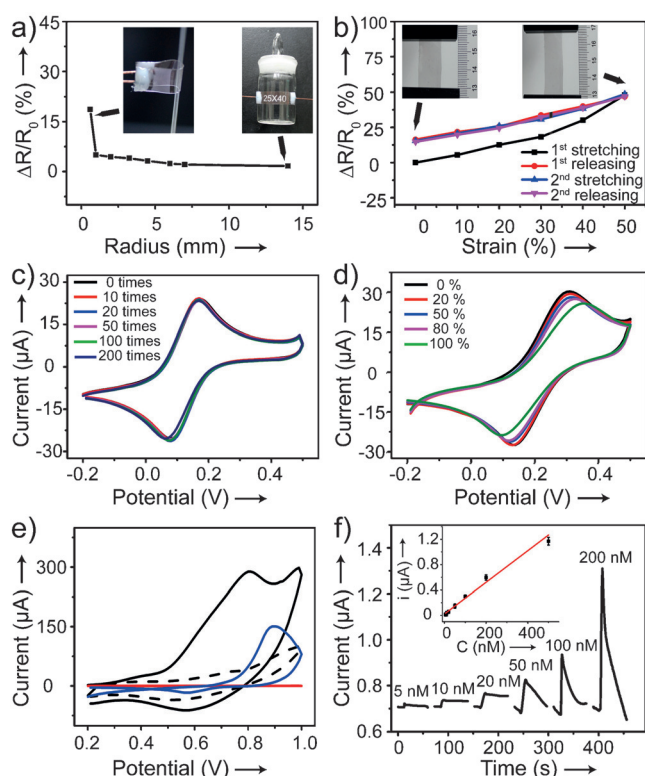


Figure 2. a) $\Delta R/R_0$ as a function of radius of curvature. The insets are images of Au NTs/PDMS film bended with minimum (left) and maximum (right) radius of curvature. b) $\Delta R/R_0$ as a function of tensile strain in the first two stretch-release cycles. The insets are images of Au NTs/PDMS film before (left) and after (right) being stretched to 50%. CVs of Au NTs/PDMS film obtained in $K_3[Fe(CN)_6]$ after recovering from being c) bended for different times (bending radius: 5 mm) and d) stretched to different tensile strains. e) CVs of Au NTs (black solid line), planar gold (blue line) and ITO (red line) electrodes in the presence of $50 \mu\text{M}$ NO in deaerated PBS solution. Black dash line is CV of Au NTs in the absence of NO. f) Amperometric response of Au NTs/PDMS (electrode area $0.5 \text{ cm} \times 1.0 \text{ cm}$) to increases concentration of NO at a potential of $+0.85 \text{ V}$ (vs. Ag/AgCl). The inset is the calibration curve.

stretched back, Au NTs will contact with others again, leading to partial recovery of conductivity (Figure S8b). For the subsequent cycle, $\Delta R/R_0$ was nearly identical for the same strain, indicating that a stable conductive pathway has been established after the first cycle. This was further verified by unobvious difference in $\Delta R/R_0$ when the film was stretched up to 50% or bended with a radius of 1 mm for 1000 cycles (Figure S9).

CVs of Au NTs/PDMS film were collected after subjected harsh condition including being circularly bended to radius of 1 mm and stretched to different tensile strains. The peak current and potential of ferricyanide show high repeatability (Figure 2c,d), demonstrating the electrochemical stability of the film against large mechanical bending and stretching. In physiological conditions, NO is closely involved in endothelial mechanotransduction to modulate numerous aspects of vascular homeostasis. The production of NO is dramatically influenced by mechanical forces including circumferential stretch and fluid shear stress.^[15] Therefore, it is of great

significance to real-time monitor NO level from mechanically sensitive ECs and blood tissues using deformable devices. Compared with ITO and planar gold electrode with the same geometric area, the CV of Au NTs/PDMS to NO shows a clear oxidation peak at 0.80 V (Figure 2e) with much higher current, exhibiting excellent catalytic activity to NO oxidation. This may be ascribed that the electrode is composed of nanometer-sized Au NTs, and Au nanostructures have inherent electrocatalytic performance for NO.^[12b,c] Obvious increase in current was detectable even evoked by 5 nM NO (Figure 2f), and the calculated detection limit was 3 nM ($S/N=3$), demonstrating the excellent electrochemical sensing ability.

Nanostructured substrates have been validated to affect cellular behavior for their similar dimensions with cellular surface components and extracellular matrix.^[16] It was apparently observed that HUVECs cultured on Au NWs/PDMS film for 1 h with fully outspread pseudopodia attached to the surface of the nanotubes networks (Figure 3a), and cells cultured for 3 h have recovered their characteristic spindled shapes (Figure 3b and Figure S10a). This indicates that Au NTs are quite biocompatible and can greatly promote cell adhesion. After being cultured for 72 h, the cells

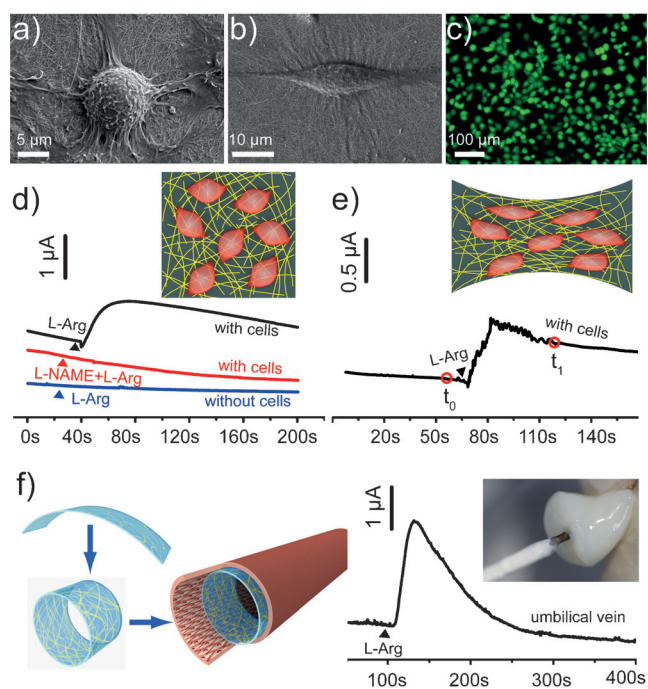


Figure 3. SEM images of HUVECs cultured on an Au NTs/PDMS film for a) 1 h and b) 3 h. c) The microscopic images of the Calcein-AM (green) and PI (red) stained HUVECs cultured on Au NTs/PDMS. Monitoring NO release from HUVECs by Au NTs/PDMS film d) without stretching and e) with stretching cycles. The timing of t_0 and t_1 were the beginning and ending of stretching cycles with a frequency of 0.5 Hz . The insets are the illustration showing the state of HUVECs and Au NTs/PDMS electrode in (d) and (e), respectively. f) Schematic illustration of the Au NTs/PDMS film inserting inside human umbilical vein process (left) and the corresponding amperometric response for NO release (right). The inset shows the experimental photograph of Au NTs/PDMS film inserting inside umbilical vein with the aid of a glass capillary.

proliferated well and covered almost all over the electrode (Figure S10b), and the cells were almost clearly alive (Figure 3c).

The Au NTs/PDMS electrode was then used to monitor NO release from living cells cultured thereon. After stimulating HUVECs with L-arginine (L-Arg) that can be enzymatically oxidized by nitric oxide synthase (NOS) to produce NO in ECs,^[17] the ampere-current therewith rose (Figure 3d, black line). Control experiments (without cells or stimulated cells with a specific NOS inhibitor, L-NAME) further verified that the increase in current was evoked by NO release (Figure 3d, blue and red lines). To test its competence as a stretchable electrochemical sensor, HUVECs were cultured on the Au NTs/PDMS electrode that was mounted on a sliding bracket, and the electrode was stretched to ca. 27% with a frequency of 0.5 Hz (cells subjected to stretching were shown in Figure S11). NO release from stretching HUVECs was preliminarily observed when cells were stimulated (Figure 3e), showing satisfying electrochemical performance of Au NTs/PDMS as a stretchable sensor in biological detection.

To break through the barrier of conventional hard and planar electrode for curvilinear tissues detections, we rolled up the Au NTs/PDMS film into a cylinder and inserted it closely into a segment of human umbilical vein (as illustrated in Figure 3f, digital images of Au NTs/PDMS film on a glass capillary and the umbilical cord were shown in Figure S12). Inside the vein the surface-compliant Au NTs/PDMS film spread and interfaced with the lining intimately, and NO release was successfully monitored from endothelial cells of umbilical vein.

To summarize, a stretchable electrochemical sensor based on Au NTs percolating networks achieved real-time monitoring of mechanically sensitive cells and tissues by deformable device. In this work, we demonstrate the capability of presented stretchable sensor in detection of NO generation from stretched ECs and inner lining of human umbilical veins. Quantitative study on the NO production from ECs and blood vessels under various kinds of mechanical stimuli is now in progress in our group. It is anticipated that this kind of stretchable sensor could be developed as a powerful tool to monitor mechanotransduction process in living cells and tissues. Further considering the convenience for preparation of Au NTs and their excellent biocompatibility, functionalization of the Au NTs with rational design will broaden its application as stretchable devices to meet the various demands in biology.

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- [1] a) D.-H. Kim, N. Lu, R. Ma, Y.-S. Kim, R.-H. Kim, S. Wang, J. Wu, S. M. Won, H. Tao, A. Islam, *Science* **2011**, *333*, 838–843; b) S. J. Benight, C. Wang, J. B. Tok, Z. Bao, *Prog. Polym. Sci.* **2013**, *38*, 1961–1977; c) S. R. Gutbrod, M. S. Sulkin, J. A. Rogers, I. R. Efimov, *Prog. Biophys. Mol. Biol.* **2014**, *115*, 244–251.
- [2] a) J. A. Rogers, T. Someya, Y. Huang, *Science* **2010**, *327*, 1603–1607; b) T. Sekitani, T. Someya, *Adv. Mater.* **2010**, *22*, 2228–2246; c) M. Park, J. Park, U. Jeong, *Nano Today* **2014**, *9*, 244–260; d) S. Yao, Y. Zhu, *Adv. Mater.* **2015**, *27*, 1480–1511; e) T. Cheng, Y. Zhang, W. Y. Lai, W. Huang, *Adv. Mater.* **2015**, *27*, 3349–3376.
- [3] a) T. Someya, Y. Kato, T. Sekitani, S. Iba, Y. Noguchi, Y. Murase, H. Kawaguchi, T. Sakurai, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 12321–12325; b) S. C. Mannsfeld, B. C. Tee, R. M. Stoltenberg, C. V. H. Chen, S. Barman, B. V. Muir, A. N. Sokolov, C. Reese, Z. Bao, *Nat. Mater.* **2010**, *9*, 859–864; c) L. Xu, S. R. Gutbrod, A. P. Bonifas, Y. Su, M. S. Sulkin, N. Lu, H.-J. Chung, K.-I. Jang, Z. Liu, M. Ying, *Nat. Commun.* **2014**, *5*, 3329.
- [4] a) R. M. Wightman, *Science* **2006**, *311*, 1570–1574; b) A. Schulte, W. Schuhmann, *Angew. Chem. Int. Ed.* **2007**, *46*, 8760–8777; *Angew. Chem.* **2007**, *119*, 8914–8933; c) C. Amatore, S. Arbault, M. Guille, F. Lemaitre, *Chem. Rev.* **2008**, *108*, 2585–2621; d) K. L. Adams, M. Puchades, A. G. Ewing, *Annu. Rev. Anal. Chem.* **2008**, *1*, 329–355; e) G. S. Wilson, M. A. Johnson, *Chem. Rev.* **2008**, *108*, 2462–2481; f) M. L. Rogers, M. G. Bouteille, *Annu. Rev. Anal. Chem.* **2013**, *6*, 427–453; g) P. Yu, X. He, L. Mao, *Chem. Soc. Rev.* **2015**, *44*, 5959–5968.
- [5] a) C. Hahn, M. A. Schwartz, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 53–62; b) T. Zhou, Y. Zheng, J. Qiu, J. Hu, D. Sun, C. Tang, G. Wang, *J. Mech. Med. Biol.* **2014**, *14*, 1430006, 1–31.
- [6] a) E. Hecht, P. Knittel, E. Felder, P. Dietl, B. Mizaikoff, C. Kranz, *Analyst* **2012**, *137*, 5208–5214; b) E. Hecht, A. Liedert, A. Ignatius, B. Mizaikoff, C. Kranz, *Biosens. Bioelectron.* **2013**, *44*, 27–33.
- [7] a) J. R. Windmiller, J. Wang, *Electroanalysis* **2013**, *25*, 29–46; b) A. J. Bandodkar, W. Jia, J. Wang, *Electroanalysis* **2015**, *27*, 562–572.
- [8] A. J. Bandodkar, R. Nuñez-Flores, W. Jia, J. Wang, *Adv. Mater.* **2015**, *27*, 3060–3065.
- [9] a) K. S. Kim, Y. Zhao, H. Jang, S. Y. Lee, J. M. Kim, K. S. Kim, J.-H. Ahn, P. Kim, J.-Y. Choi, B. H. Hong, *Nature* **2009**, *457*, 706–710; b) T. Sekitani, H. Nakajima, H. Maeda, T. Fukushima, T. Aida, K. Hata, T. Someya, *Nat. Mater.* **2009**, *8*, 494–499; c) M.-S. Lee, K. Lee, S.-Y. Kim, H. Lee, J. Park, K.-H. Choi, H.-K. Kim, D.-G. Kim, D.-Y. Lee, S. Nam, *Nano Lett.* **2013**, *13*, 2814–2821.
- [10] a) S. De, T. M. Higgins, P. E. Lyons, E. M. Doherty, P. N. Nirmalraj, W. J. Blau, J. J. Boland, J. N. Coleman, *ACS Nano* **2009**, *3*, 1767–1774; b) L. Hu, H. S. Kim, J.-Y. Lee, P. Peumans, Y. Cui, *ACS Nano* **2010**, *4*, 2955–2963; c) F. Xu, Y. Zhu, *Adv. Mater.* **2012**, *24*, 5117–5122; d) P. Lee, J. Lee, H. Lee, J. Yeo, S. Hong, K. H. Nam, D. Lee, S. S. Lee, S. H. Ko, *Adv. Mater.* **2012**, *24*, 3326–3332; e) J. Ge, H. B. Yao, X. Wang, Y. D. Ye, J. L. Wang, Z. Y. Wu, J. W. Liu, F. J. Fan, H. L. Gao, C. L. Zhang, S. H. Yu, *Angew. Chem. Int. Ed.* **2013**, *52*, 1654–1659; *Angew. Chem.* **2013**, *125*, 1698–1703.
- [11] a) Y. Sun, B. Gates, B. Mayers, Y. Xia, *Nano Lett.* **2002**, *2*, 165–168; b) B. Wiley, Y. Sun, Y. Xia, *Acc. Chem. Res.* **2007**, *40*, 1067–1076.
- [12] a) Y. X. Li, J. T. Cox, B. Zhang, *J. Am. Chem. Soc.* **2010**, *132*, 3047–3054; b) A. Yu, Z. Liang, J. Cho, F. Caruso, *Nano Lett.* **2003**, *3*, 1203–1207; c) Y.-J. Li, C. Liu, M.-H. Yang, Y. He, E. Yeung, *Electroanal. Chem.* **2008**, *622*, 103–108.

- [13] a) Y. Yoo, K. Seo, S. Han, K. S. Varadwaj, H. Y. Kim, J. H. Ryu, H. M. Lee, J. P. Ahn, H. Ihee, B. Kim, *Nano Lett.* **2010**, *10*, 432–438; b) H. Yao, J. Duan, D. Mo, H. Y. Günel, Y. Chen, J. Liu, T. Schäpers, *J. Appl. Phys.* **2011**, *110*, 094301; c) S. Ye, G. Marston, J. R. McLaughlan, D. O. Sigle, N. Ingram, S. Freear, J. J. Baumberg, R. J. Bushby, A. F. Markham, K. Critchley, *Adv. Funct. Mater.* **2015**, *25*, 2117–2127.
- [14] a) H. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith, *Science* **2007**, *318*, 426–430; b) T. Akter, W. S. Kim, *ACS Appl. Mater. Interfaces* **2012**, *4*, 1855–1859.
- [15] a) D. Harrison, J. Widder, I. Grumbach, W. Chen, M. Weber, C. Searles, *J. Intern. Med.* **2006**, *259*, 351–363; b) H.-J. Hsieh, C.-A. Liu, B. Huang, A. H. Tseng, D. L. Wang, *J. Biomed. Sci.* **2014**, *21*, 1–15.
- [16] a) R. Flemming, C. Murphy, G. Abrams, S. Goodman, P. Nealey, *Biomaterials* **1999**, *20*, 573–588; b) A.-S. Andersson, J. Brink, U. Lidberg, D. S. Sutherland, *IEEE Trans. Nanobiosci.* **2003**, *2*, 49–57; c) I. Smith, X. Liu, L. Smith, P. Ma, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2009**, *1*, 226–236.
- [17] a) R. M. Palmer, D. Ashton, S. Moncada, *Nature* **1988**, *333*, 664–666; b) Y.-L. Liu, X.-Y. Wang, J.-Q. Xu, C. Xiao, Y.-H. Liu, X.-W. Zhang, J.-T. Liu, W.-H. Huang, *Chem. Sci.* **2015**, *6*, 1853–1858.

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