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Meng Xu, Sayantan Pradhan, Ramendra K. Pal, Vamsi K. Yadavalli, "Fully organic, flexible, and biodegradable components for bioinspired electronics," Proc. SPIE 10982, Micro- and Nanotechnology Sensors, Systems, and Applications XI, 109821Q (13 May 2019); doi: 10.1117/12.2517804

SPIE.

Event: SPIE Defense + Commercial Sensing, 2019, Baltimore, Maryland, United States

Invited Paper

Fully organic, flexible, and biodegradable components for bioinspired electronics

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ABSTRACT

Advances in the field of nature-inspired/derived biomaterials have been revolutionizing the production of next-generation biomedical devices over the past few years, and will continue to make impacts in the field. Of special interest is the application of biodegradable materials in the fabrication of fully organic, intrinsically flexible, thin film devices. Components with precisely patterned micro- or nano-scale circuits can provide different functions as microelectrodes, biosensors, and supercapacitors. Advantages include the ability to provide conformal contact at non-planar biointerfaces, being able to be degraded at controllable rate, and invoking minimal reactions within the body. These factors present great potential as implantable devices for *in-vivo* applications, while also addressing concerns with "electronic waste" by being intrinsically degradable. The fabrication of such flexible bioelectronics requires a careful optimization of mechanical properties, electrical conductivity, and precise fabrication using materials that are often not easily adapted to such processes. One option of particular interest is the construction of biocompatible and biodegradable, flexible bioelectronics based on silk proteins. In this work, we present the combination of photo-crosslinkable silk proteins and conductive polymers to precisely fabricate flexible devices for the sensing of different targets of interest. A facile and scalable photolithography is applied to fabricate flexible substrates with conductive micropatterns which show tunable electrical and mechanical properties. Competitive conductivity, as well as excellent biocompatibility and controllable biodegradability are shown. Through this work, the possibility of making next-generation, fully organic, flexible bioelectronics is explored.

Keywords: Silk Proteins, Conductive Polymers, Photolithography, Flexible Bioelectronics, Biosensing

1. INTRODUCTION

The evolution of bioelectronics is at a transitional point in which research has shifted from the development of rigid electronic components to the fabrication of flexible replacements [1, 2]. Next-generation flexible bioelectronics are expected to have a broad spectrum of biomedical applications, including electronic skins [3], soft robotics [4], tissue engineering [5], and particularly wearable or implantable biosensors, for the continuous monitoring of human bio-information [6-8]. Such flexible systems have attracted increasing attention due to their potential to bring huge impact on the healthcare, disease diagnostics and therapeutics, and even general human way of life. Bioelectronics components are typically built on supporting substrates that are mechanically compliant, with electrochemical mechanisms that can maintain their duty of signal transduction even under motion or physical deformation. In comparison to their rigid counterparts, the primary purpose of flexible (bio)electronics is to function with minimal disturbance at the biointerfaces that are typically non-planar, soft, or non-stationary. This advantageous feature enables one to directly deploy the devices direct at the sites of interest without interrupting the normal activities of people.

However, the fabrication of these flexible bioelectronics has been limited by the selection of materials. To date, the field of flexible bioelectronics is dominated by inorganic/organic compounds based on carbon materials, metal oxide semiconductors, and polymers [9]. While these conventional materials have excellent electronic properties, they often exhibit limited compliance at biointerfaces, restricted biocompatibility, and poor environmental sustainability [9]. On the contrary, nature-derived biomaterials possess exceptional characteristics including biodegradability, biocompatibility,

Micro- and Nanotechnology Sensors, Systems, and Applications XI, edited by Thomas George, M. Saif Islam, Proc. of SPIE Vol. 10982, 109821Q · © 2019 SPIE
CCC code: 0277-786X/19/$18 · doi: 10.1117/12.2517804

water processability, low cost, non-toxicity and eco-friendliness, which can well satisfy the needs to build flexible bioelectronics for on-person health-monitoring applications. However, a challenge has often been the ability to combine high-throughput and high-resolution fabrication techniques with such materials, often restricting their use in bioelectronics and optics, beyond functioning as substrates. Here, we posit that biomimetic materials can function not only as support and structural materials, but as functional components of bioelectronics devices, thereby resulting in fully organic devices. In particular, we focus on silk proteins from the silkworm as materials of choice.

The proteins fibroin and sericin, as natural biomaterials obtained from *Bombyx mori* silkworm cocoons, have been recognized as a very appealing biocompatible and regeneratable resource and fostered various applications in tissue engineering and regenerative medicine [10]. Extensive research has utilized these silk proteins due to their low cost and sustainability, coupled with excellent mechanical strength, biocompatibility, controllable degradability, and ease processability into versatile structural formats [11, 12]. In particular, our group has shown biochemically modified variants of fibroin and sericin that permit their use with techniques such as photolithography. Fabrication techniques using printing, molding, imprinting, and photolithography have shown silk proteins for the construction of flexible optical, photonic, optoelectronic, and electronic devices [13-15]. However, there remain several technical challenges that need to be addressed. For instance, a direct patterning with high fidelity and complex silk-based functional features for the integration of electronic systems is an ongoing challenge. Furthermore, the fabrication of flexible bioelectronics may require compositions with metals, nanowires, or conductive polymers in order to provide the electrical transduction. This requires careful optimization of various properties so that the intrinsic advantages can still be maintained, while providing the overall signal sensitivity and electrochemical properties All these challenges require further improvement for the fabrication of silk-based flexible bioelectronics so that they can serve as better alternatives to their inorganic counterparts.

In this work, we explore the fabrication of different types of fully organic, flexible, and biodegradable bioelectronic components based on photocrosslinkable silk proteins fibroin and sericin, and the conductive polymer, poly(3,4-ethylenedioxythiophene): poly(styrene sulfonate) (PEDOT:PSS). We specifically focus on their use as biosensor elements in which the entire device is organic. A rapid and scalable photolithographic technique is used in the fabrication of flexible substrates with conductive micropatterns as microelectrodes. This technique is simple and requires no clean room or harsh treatment, and allows rapid engineering of various topographic electrical systems over relatively large surfaces. The developed organic flexible bioelectronic components are demonstrated to be biocompatible to support cell adhesion and proliferation. The silk based biocomposite conducting ink functions as a structural matrix that can entrap the water dispersible PEDOT: PSS and other biorecognition elements such as enzymes and antibodies. The biosensing platforms constructed based on this silk proteins/conductive polymer system were not only able to monitor common electrochemically active small molecules such as glucose, ascorbic acid, and dopamine, they were also used to detect larger biomarkers such as proteins. Additionally, the developed flexible bioelectronics were based on all organic materials, allowing them to show fully biodegradability via proteolysis. Through more engineering designs, we can further explore the options of using this silk proteins/conductive polymer system to fabricate various types of flexible bioelectronics and construct fully organic integrated electronic platforms.

2. EXPERIMENTAL SECTION

2.1 Synthesis of photocrosslinkable silk proteins

Silk fibroin was extracted from silk cocoons using a standard protocol developed elsewhere [16]. Photoreactive fibroin and sericin were prepared by introducing photoreactive moieties to the protein side chains as reported earlier [17]. Pure fibroin or sericin obtained was dissolved in 1 M LiCl/ dimethylsulfoxide (DMSO) and reacted with 2-Isocyanatoethyl methacrylate in stoichiometric amounts for 5 hours at 60^{o}C while maintaining inert conditions using a constant flow of nitrogen. After the completion of the reaction, the product mixture was added to cold ethanol and the methacrylated proteins were obtained as the precipitate. The methacrylated proteins were repeatedly washed with a 1:1 ratio of cold ethanol and acetone followed by centrifugation and then lyophilized for 24 hours. Both photoreactive fibroin and sericin were obtained as dry powder and stored at room temperature before use.

2.2 Fabrication of fibroin substrates

The fibroin substrate was fabricated by dissolving 7.5% (w/v) of photoreactive fibroin in formic acid (Acros Organics 98%), and 2.5% (w/v) photo initiator (Irgacure 2959, BASF) was added to the fibroin solution. The solution was drop-casted on clean glass slides and air dried for 15 to 20 minutes in order to evaporate the excess formic acid. The samples were exposed under a 365 nm UV lamp (Lumen Dynamics OmniCure 1000 system) for 1.5 seconds at 2000 mW cm^{-2} for cross-linking. To obtain free standing fibroin films, the samples were dipped in DI water which facilitated the delamination of the fibroin films from the glass.

2.3 Fabrication of conductive patterns on flexible substrates

1% (wt/vol.) dispersion of PEDOT: PSS (Orgacon™, Sigma-Aldrich, St. Louis, MO) in water was obtained through ultra-sonication for 20 minutes. The PEDOT: PSS dispersion was filtered and 5% DMSO (v/v) was added to get the final stock solution. The basic conductive ink containing 2.5% (w/v) of photoreactive sericin, 1% (w/v) PEDOT: PSS, and 0.5% (v/v) of photoinitiator (Darocur 1173, Ciba Specialty Chemicals Inc., Basel, Switzerland) was then casted on the FPP substrates and air dried in the dark. Micropatterns were formed by exposure to UV light through a chrome photomask for 1.5 seconds. The patterns were developed in deionized water to obtain flexible bioelectronic components (**Figure 1**).

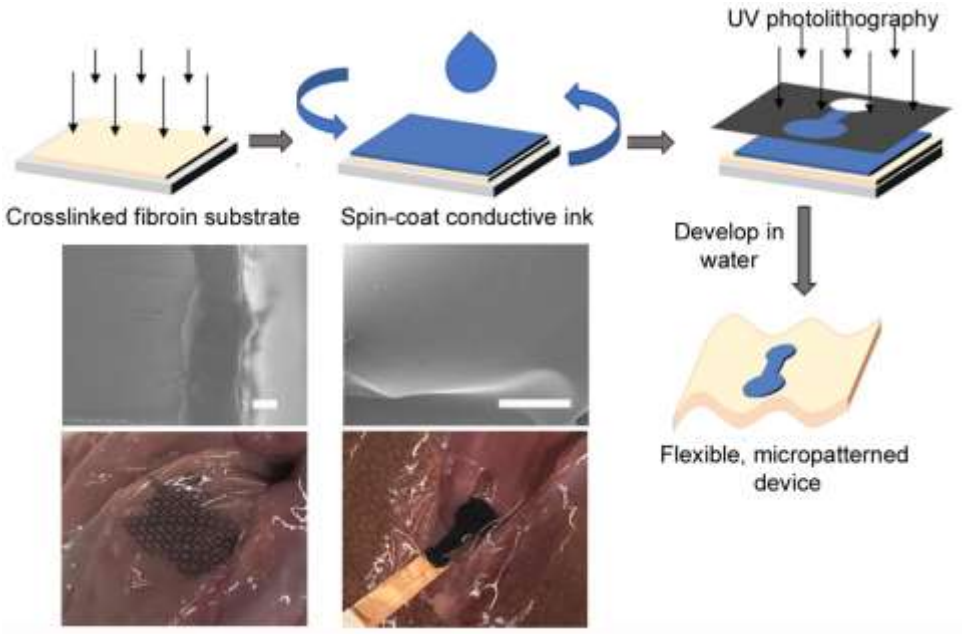

Figure 1: Schematic showing the fabrication of flexible devices based on silk proteins. SEM images showing surfaces of electrodes (scale bar = 1 um). The flexible devices can be conformably placed on biological interfaces for the detection of various analytes.

3. RESULTS AND DISCUSSION

Advancements in the development of bioelectronics components depends strongly on the use of substrate materials that can support an ensemble of functionalities, while being adaptable to high resolution microfabrication techniques [18]. Commercially available materials such as polydimethylsiloxane (PDMS) and poly(ethylene terephthalate) (PET) have been extensively explored for the realization of flexible electronics [19-21]. Recently, the use of naturally occurring biomaterials, such as silk proteins have been proposed as substrate materials in bioelectronics [22]. Such materials provide unique advantages including sustainable, green-processing, biocompatibility and biodegradability. We argue that such proteins can also be used to fabricate the functional elements of bioelectronics components. We have earlier demonstrated that silk fibroin and sericin, the core proteins in silk from silkworms, can be functionalized with photo-reactive moieties, which permits them to be used in photolithographic processes to form high resolution structures. This allows proteins to be used for the fabrication of various components for bioelectronics applications. In initial work, we showed how these

proteins can be formed as micro and nanostructures on conventional rigid substrates such as silicon and glass. However, these proteins can also be fabricated into mechanically flexible, optical transparent, robust and biocompatible substrates. The thickness of the films can be easily tuned by varying the amount of photoreactive fibroin solution casted on the glass slides. The obtained flexible fibroin substrates were found to be insoluble in water and stable in a wide range of solvents, ideal for application in physiological environments. The films are also found to be mechanically robust with a strength >100 MPa and minimal loss in chemical and physical properties with mechanical deformation. This allows them to be rolled up or folded in any geometry and conformation without compromising functionality. The biocompatibility profiles of these films have been observed by the adhesion and proliferation of human bone marrow-multipotent stromal cells (hBM-MSCs) (**Figure 2)**. These substrate films were also shown to be fully degradable in enzymatic conditions within few weeks [23, 24].

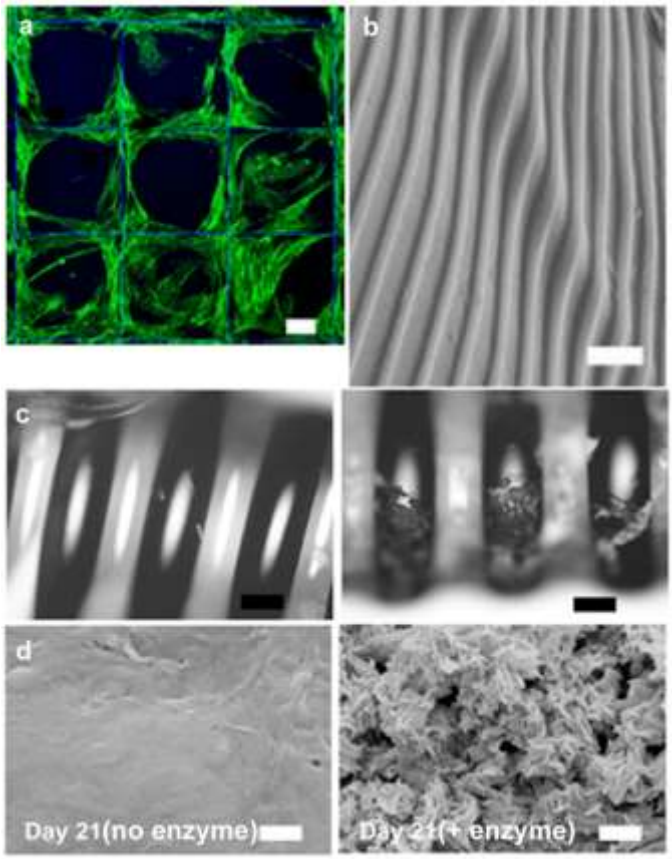

Figure 2: (a) Showing the cell guidance and cytocompatibility of the micropatterned films prepared using this technique. Human bone marrow-multipotent stromal cells (hBM-MSCs) along flexible micropatterned films such as those shown in (b). (a) Scale bar = 250 µm. (b) Scale bar = 50 µm. (c) Electrodes break down over a period of weeks in enzyme (right) but remain stable in buffer (left) (Scale bar = 1 mm). (d) SEM images showing closeup of degradation in the presence of enzyme (scale bar = 100 µm).

Although bio-derived materials possess a variety of properties that enables their use as functional materials in bioelectronics, their electrically insulating nature has limited their use as active component. We have reported a biocomposite conductive ink based on the photoreactive silk sericin and the conducting polymer PEDOT: PSS, which is suitable for the fabrication of active components in organic electronics (**Figure 1).** The photoreactive sericin is functionalized with the same photo-active moieties as the fibroin so that it can be self-crosslinked as well as covalently bound to fibroin substrates under UV light, thereby producing properly patterned bioelectronics. Unlike most naturally occurring polymers, photoreactive sericin is soluble in water, which offers great processability when used with most of the biorecognition reagents such as enzymes and antibodies. Furthermore, it also allows the easy development of patterns once immersed in water. The sericin area that was not cross-linked could be easily washed off, giving very clean, high resolution and high fidelity micropatterns, whose complexity is only limited by the engineering design of the photomasks. The entire fabrication process was done on bench-top and low cost using only benign solvents. Due to the presence of

chemical linkage, the micropatterns can withstand all kinds of mechanical deformations without any delamination or degradation in physio-chemical and electrical properties.

Upon electrochemical characterization, the conductive ink composite has been found to possess very favourable performance matrices. The conductivity of the ink has been found to increase with the increasing concentration of PEDOT: PSS in the composite. 28% PEDOT: PSS has been used as the optimal concentration in the fabrication of micropatterns as beyond that the penetration of UV light through the material will be compromised. A stable CV with in a potential window of -1.0 to 1.6 V in PBS without any water splitting confirms its potential use for the detection of important biomolecules at different oxidation and reduction potentials. Dopamine, a neurotransmitter, and ascorbic acid, commonly known as vitamin C, have been monitored using the developed organic bioelectrodes to demonstrate direct electrochemical biosensing as they are of great physiological importance. A chronoamperometric setup using patterned SPP/ PEDOT: PSS as working electrode, Ag/AgCl as the reference electrode and platinum as counter electrode was used in the electrochemical detection to separately monitor dopamine and ascorbic acid. The response curves obtained from dopamine and ascorbic acid sensing were found to be both linear in the range of 10µM to 300 µM. The sensors showed high sensitivity towards these analytes and good response time (~30 seconds) even without the addition of other conductive materials such as graphene (**Figure 3)** [25, 26]

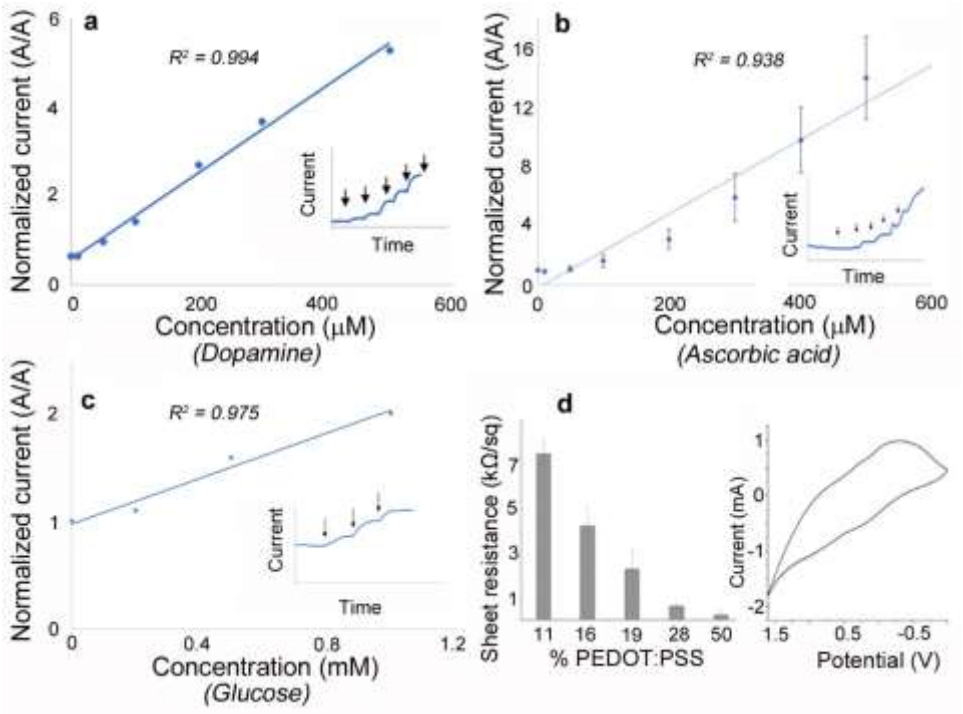

Figure 3: Calibration curves of detection of various analytes using the biosensors discussed. The insets show the respective amperometric data (arrows represent addition time points). (a) dopamine, (b) ascorbic acid and (c) glucose. The ranges are all within the physiologically relevant regimes. In the case of glucose sensing, the conducting ink contains immobilized glucose oxidase enzyme. (d) electrochemical characterization of the conducting ink leading to optimization of concentration.

The sericin in the water-based biocomposites conductive ink forms a stable biocompatible crosslinked matrix that allows the encapsulation of biomolecules, such as enzymes and antibodies. This important characteristic enables to offer these sensors the functionality for indirect sensing through enzymatic reactions or antibody binding. Encapsulation of glucose oxidase (GOx) in the conductive ink permits the realization of flexible and biocompatible glucose sensors. The oxidation of glucose was mediated by the ferrocene salt, and the electrons were shuttled and transferred through PEDOT: PSS in the patterned matrix to the sensing instrument. The amperometric response curve of this glucose sensors has been shown in **Figure 3.** The sensors show a liner response in the range of 0.2 to 1 mM with a sensitivity of 5.39 µA/mM cm^2 and a response time of ~20 seconds. The electrochemical measurement also confirms that the glucose oxidase retained its enzymatic activity throughout the fabrication process.

The expansion of target repertoire to the larger molecule biomarkers is of critical importance for clinical diagnosis and therapeutic interventions, yet less explored due to the challenges in the immobilization of biorecognition elements (antibodies, aptamers etc) and signal transduction in a flexible biosensor format. With the good biocompatibility and water processability of the sericin/PEDOT: PSS conductive ink, we also demonstrated to use the developed flexible biosensing system for the detection of one important protein biomarker, vascular endothelial growth factor (VEGF). The adaptation of electrochemical impedance spectroscopy (EIS) in this flexible biosensor enabled label-free ultrasensitive detection of VEGF in various types of media (**Figure 4**), indicating the broad suitability of the developed sensor for different applications [27]. The equivalent circuit model was used to evaluate the sensor response to the concentration of VEGF. It showed a wide dynamic detection range that can very well cover the cut-off levels of VEGF in certain cancer patients (commonly 0.1 to 1 ng/ml).

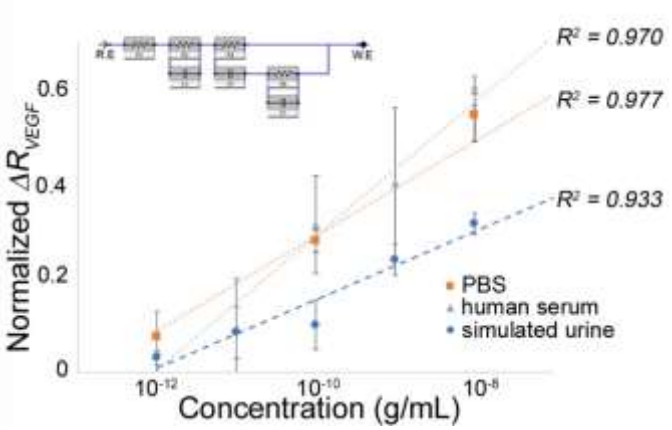

Figure 4: Detection of vascular endothelial growth factor (VEGF) using anti-VEGF antibodies immobilized in the conducting ink. This sensor uses impedimetric detection and can reliably detect low concentrations of these large protein molecules in a variety of biologically relevant fluids. The inset shows the equivalent circuit model for the system.

4. CONCLUSION

In summary, here we discuss the fabrication of fully organic and biodegradable flexible biosensors based on photoreactive silk proteins and the conductive polymer PEDOT: PSS. The applications of biosensing using the developed electronic system for various types of biological targets is explored. The sensor was fabricated using a simple and eco-friendly photolithography technique. The organic microelectrodes made from silk proteins and PEDOT: PSS are not only able to offer electrochemical functionality by themselves and be used in a free-standing format to directly attach to biointerfaces, they are also capable of providing supporting frame for the immobilization of biofunctional molecules such as enzymes and antibodies and the protection for these delicate bioreceptors. The broad adaptability, flexible and conformable thin film format, good biocompatibility, biodegradability, light-weight and low-cost of these fully organic silk-based devices enable promising point-of-care, in situ, or in vivo applications for healthcare.

REFERENCES

[1] Someya, T., Kaltenbrunner, M., Yokota, T., "Ultraflexible organic electronics", MRS Bulletin 40(12), 1130-1137 (2015).
[2] Sekitani, T., Zschieschang, U., Klauk, H., Someya, T., "Flexible organic transistors and circuits with extreme bending stability", Nat. Mater. 9, 1015 (2010).
[3] Wang, S., Xu, J., Wang, W., Wang, G.-J.N., Rastak, R., Molina-Lopez, F., Chung, J.W., Niu, S., Feig, V.R., Lopez, J., Lei, T., Kwon, S.-K., Kim, Y., Foudeh, A.M., Ehrlich, A., Gasperini, A., Yun, Y., Murmann, B., Tok, J.B.H., Bao, Z., "Skin electronics from scalable fabrication of an intrinsically stretchable transistor array", Nature 555, 83 (2018).
[4] Rus, D., Tolley, M.T., "Design, fabrication and control of soft robots", Nature 521(7553), 467-475 (2015).

[5] Balint, R., Cassidy, N.J., Cartmell, S.H., "Conductive polymers: Towards a smart biomaterial for tissue engineering", Acta Biomaterialia 10(6), 2341-2353 (2014).
[6] Chen, Y., Lu, S., Zhang, S., Li, Y., Qu, Z., Chen, Y., Lu, B., Wang, X., Feng, X., "Skin-like biosensor system via electrochemical channels for noninvasive blood glucose monitoring", Sci. Adv. 3(12), e1701629 (2017).
[7] Yang, Y., Yang, X., Tan, Y., Yuan, Q., "Recent progress in flexible and wearable bio-electronics based on nanomaterials", Nano Research 10(5), 1560-1583 (2017).
[8] Xu, M., Obodo, D., Yadavalli, V.K., "The design, fabrication, and applications of flexible biosensing devices", Biosens. Bioelectron. 124-125, 96-114 (2018).
[9] Wang, L., Chen, D., Jiang, K., Shen, G., "New insights and perspectives into biological materials for flexible electronics", Chem. Soc. Rev. 46(22), 6764-6815 (2017).
[10] Tao, H., Kaplan, D.L., Omenetto, F.G., "Silk Materials - A Road to Sustainable High Technology", Adv. Mater., 24(210), 2824-2837 (2012).
[11] Rockwood, D.N., Preda, R.C., Yucel, T., Wang, X.Q., Lovett, M.L., Kaplan, D.L., "Materials fabrication from Bombyx mori silk fibroin", Nature Protocols 6, 1612 (2011).
[12] Zhu, B., Wang, H., Leow, W.R., Cai, Y., Loh, X.J., Han, M.-Y., Chen, X., "Silk Fibroin for Flexible Electronic Devices", Adv. Mater. 28(22), 4250-4265 (2016).
[13] Koh, L.-D., Cheng, Y., Teng, C.-P., Khin, Y.-W., Loh, X.-J., Tee, S.-Y., Low, M., Ye, E., Yu, H.-D., Zhang, Y.-W., Han, M.-Y., Structures, mechanical properties and applications of silk fibroin materials, Prog. Polym. Sci. 46. 86-110 (2015).
[14] Tansil, N.C., Koh, L.D., Han, M.-Y., "Functional Silk: Colored and Luminescent", Adv. Mater. 24(11), 1388-1397 (2012).
[15] Kim, D.-H., Viventi, J., Amsden, J.J., Xiao, J., Vigeland, L., Kim, Y.-S., Blanco, J.A., Panilaitis, B., Frechette, E.S., Contreras, D., Kaplan, D.L., Omenetto, F.G., Huang, Y., Hwang, K.-C., Zakin, M.R., Litt, B., Rogers, J.A., "Dissolvable films of silk fibroin for ultrathin conformal bio-integrated electronics", Nat. Mater. 9(6), 511-517 (2010).
[16] Rockwood, D.N., Preda, R.C., Yucel, T., Wang, X.Q., Lovett, M.L., Kaplan, D.L., "Materials fabrication from Bombyx mori silk fibroin", Nat. Protoc. 6(10), 1612-1631 (2011).
[17] Kurland, N.E., Dey, T., Kundu, S.C., Yadavalli, V.K., "Precise Patterning of Silk Microstructures Using Photolithography", Adv. Mater. 25(43), 6207-6212 (2013).
[18] Bettinger, C.J., Bao, Z., "Biomaterials-based organic electronic devices", Polym. International 59(5) (2010) 563-567.
[19] Pal, R.K., Pradhan, S., Narayanan, L., Yadavalli, V.K., "Micropatterned conductive polymer biosensors on flexible PDMS films, Sens. Actuators B-Chem. 259, 498-504 (2018).
[20] Vosgueritchian, M., Lipomi, D.J., Bao, Z., "Highly Conductive and Transparent PEDOT:PSS Films with a Fluorosurfactant for Stretchable and Flexible Transparent Electrodes", Adv. Funct. Mater. 22(2), 421-428 (2012).
[21] Zardetto, V., Brown, T.M., Reale, A., Di Carlo, A., "Substrates for flexible electronics: A practical investigation on the electrical, film flexibility, optical, temperature, and solvent resistance properties", J. Polym. Sci. B: Polym. Phys. 49(9), 638-648 (2011).
[22] Kim, D.-H., Kim, Y.-S., Amsden, J., Panilaitis, B., Kaplan, D.L., Omenetto, F.G., Zakin, M.R., Rogers, J.A., "Silicon electronics on silk as a path to bioresorbable, implantable devices", Appl. Phys. Lett. 95(13), 133701 (2009).
[23] Pal, R.K., Kundu, S.C., Yadavalli, V.K., "Fabrication of flexible, fully organic, degradable energy storage devices using silk proteins", ACS Appl. Mater. Inter. 10(11), 9620-9628 (2018).
[24] Xu, M., Pradhan, S., Agostinacchio, F., Pal, R.K., Greco, G., Mazzolai, B., Pugno, N.M., Motta, A., Yadavalli, V.K., "Easy, Scalable, Robust, Micropatterned Silk Fibroin Cell Substrates", Adv. Mater. Inter. 1801822, (2019).
[25] Pal, R.K., Farghaly, A.A., Collinson, M.M., Kundu, S.C., Yadavalli, V.K., "Photolithographic Micropatterning of Conducting Polymers on Flexible Silk Matrices", Adv. Mater. 28(7), 1406-1412 (2016).
[26] Pal, R.K., Farghaly A.A., Wang, C., Collinson, M.M., Kundu , S.C., Yadavalli, V.K., "Conducting polymer-silk biocomposites for flexible and biodegradable electrochemical sensors", Biosens. Bioelectron. 81, 294-302 (2016).
[27] Xu, M., Yadavalli, V.K., "Flexible biosensors for the impedimetric detection of protein targets using silk-conductive polymer biocomposites", ACS Sensors, in press (2019).