ADVANCED HEALTHCARE MATERIALS
www.advhealthmat.de

Microneedle Electrodes Toward an Amperometric Glucose-Sensing Smart Patch

*Michael A. Invernale, Benjamin C. Tang, Royce L. York, Long Le, David Yupeng Hou, and Daniel G. Anderson**

Currently, diabetes affects over 285 million people worldwide, and is predicted to impact 1 in 10 individuals by the year 2030, according to the World Health Organization.[1] The American Diabetes Association states that type 1 and type 2 diabetes affects over 25 million people alone in the United States.[2] Long-term, accurate sensing of blood glucose concentration is a major concern for diabetic patients,[3] and satisfactory compliance with testing is difficult to achieve, given the painful, repetitive nature of the commonly used finger-prick method.[4] Efforts for longer-term, implantable sensors require the development of highly sensitive, fast-responding, accurate, and biocompatible-sensing elements. A recent review by Bratlie et al.[5] describes the various current approaches, therapies, and state of the art technologies in this field. Much effort has been focused toward implantable, continuous sensors, with some commercial success.[6] Currently, the state of the art continuous glucose-sensing devices suffer from poor and variable lifetimes, precluding FDA approval for independent glucose sensing without supplemental blood pricks.[7] Furthermore, these are single-sensor systems with no redundancy for signal averaging or failure detection or replacement. Continuous sensors, such as the DexCom SEVEN+ system, must be frequently changed and have a bulky external component.[6]

Microneedle-based arrays can be utilized for a variety of therapeutic and diagnostic systems.[8] They have generated significant interest, owing to their potential for painless sampling, and delivery to the intradermal space.[9] To date, microneedles have been used for the injection of insulin,[10–13] and as sensors for a variety of analytes.[14–19] There have been attempts to use microneedles as a means of drawing blood or other fluids for amperometric glucose sensing using a separate sensor array.[20–23] These sensors are not in direct contact with interstitial fluid, but are instead making the use of capillary action to draw out analyte in a new way. Here, we develop microneedles to address the current limitations in glucose-sensing devices. We develop a prototype microneedle-based electrode system for the amperometirc detection of glucose that uses the needle itself as the functional electrode array. Our system is an example of a direct-contact sensor array that does not rely on a separately built sensor architecture; rather it is a more elegant and simple design that is capable of providing simple, painless, redundant, and continuous glucose-monitoring systems (CGMS) for diabetic patients.

Toward that end, we sought to develop a microneedle-based "Smart Patch" sensor platforms for painless, continuous intradermal sensing. **Figure 1** shows images of the microneedle arrays used in this study, the schematic design of our sensor approach, and an envisioned "Smart Patch" based on this technology. We utilized conducting polymers, such as poly(3,4-ethylenedioxythiophene) (PEDOT), as electrical mediators for the sensing of glucose, as well as an immobilization agent for the glucose-specific enzyme, glucose oxidase (GOx). GOx, currently used in blood glucose strips, converts glucose (consuming oxygen) into gluconic acid (produces hydrogen peroxide). Flavin adenosine dinucleotide (FAD) cofactor, associated with GOx, undergoes reversible oxidation and reduction during this process. As glucose is converted, the associated current produced can be sensed by applying a voltage. Using GOx immobilized in PEDOT, this signal can be transduced at safe low voltages, affording accurate and fast responses to physiological changes in glucose concentration. Similar sensing mechanisms have been employed in the past,[24] and other systems may also work on this microneedle platform; for this study, we chose the conducting polymer architecture due to its unique advantages toward immobilization and signal transduction without loss of signal over time, unlike small-molecule mediators, like quinones. Conducting polymers swell slightly in aqueous media, as compared with organic solvents. This swelling is sufficient to allow the diffusion of glucose to the enzyme for signal transduction but is not sufficient to allow for the escape of the large enzyme from the polymer film. In this way, the immobilized enzyme is kept stable and does not denature or contort, thus losing its function.[24]

Cytotoxicity and biocompatibility are among the largest issues facing such sensors, as poorly compliant materials result in unwanted inflammation, fouling, and other adverse physiological effects. Here, we investigate the potential of conducting

Dr. M. A. Invernale, Dr. B. C. Tang, R. L. York, Prof. D. G. Anderson
The David H. Koch Institute for Integrative Cancer Research
Massachusetts Institute of Technology
500 Main Street, Cambridge, MA 02142, USA
E-mail: dgander@mit.edu
Dr. M. A. Invernale, Dr. B. C. Tang, R. L. York, Prof. D. G. Anderson
Department of Anesthesiology
Children's Hospital Boston
300 Longwood Avenue, Boston, MA, 02115, USA
L. Le, D. Y. Hou, Prof. D. G. Anderson
Department of Chemical Engineering
Massachusetts Institute of Technology
77 Massachusetts Avenue, Rm. 66–350, Cambridge, MA 02139, USA
Prof. D. G. Anderson
Harvard-MIT Division of Health Science Technology
Massachusetts Institute of Technology
45 Carleton Street, Building E25–342, Cambridge, MA 02142, USA

DOI: 10.1002/adhm.201300142

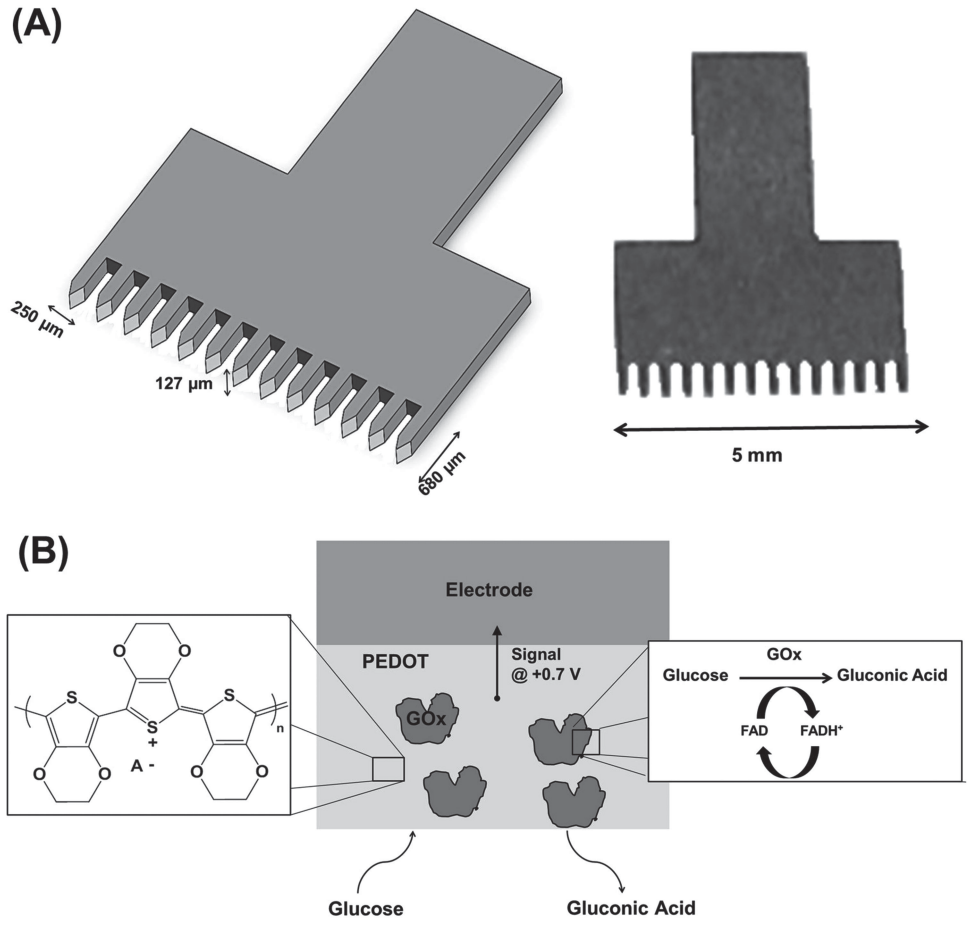

Figure 1. A) Image of the microneedle arrays. B) Sensor design schematic.

polymers for glucose sensors, as well as various film parameters as they pertain to stability and accuracy. The final sensor design is comprised of a platinum-coated stainless steel in-line 2D microneedle array coated with a film of PEDOT in which GOx has been immobilized. These sensors have proven to be efficacious within the physiological range of 0–432 mg dL^{-1} (0–24 × 10^{-3} M) glucose (**Figure 2**). The average healthy blood glucose level for a patient is 100 mg dL^{-1} (5.6 × 10^{-3} M) and current finger-prick test strip sensors are rated from 20 to 500 mg dL^{-1} (1.1–27.8 × 10^{-3} M).[25] These results suggest that a conducting polymer-based continuous glucose-monitoring patch is a realistic platform for diabetes theranostics.

PEDOT was electrochemically polymerized on 316L grade stainless steel and platinum microneedles in the presence of GOx, resulting in thin films composed of GOx immobilized within an electrically conducting polymer matrix. The conducting film acts as a mediator for transducing the signal generated by the enzymatic oxidation of glucose. By applying a +0.7 V potential bias with respect to a Ag/AgCl reference electrode, changes in amperometric response can be observed upon successive additions of glucose (Figure 2). These signals were then correlated to a concentration of glucose and a linear curve across the physiologically relevant range was generated (Figure 2).

We tested the sensors in phosphate-buffered saline (PBS) using sequential additions of glucose, and measured current response at +0.7 V. A linear response was observed with increasing glucose concentrations (Figure 2; $R^2 > 0.97$ for all coatings). The platinum button control sensor was able to sense in the physiological glucose range of 36–468 mg dL^{-1} (2–26 × 10^{-3} M, $S/N = 9.0$). Steel microneedles were found to have significantly lowered efficacy, however, they still functioned linearly between 72 and 216 mg dL^{-1} glucose (4–12 × 10^{-3} M, $S/N = 33.8$). Once coated with platinum, sensor performance returned, as expected, to nearly the same performance as the control. Platinum-coated needles exhibited linearity between 36 and 432 mg dL^{-1} glucose (2–24 × 10^{-3} M, $S/N = 10.7$). Other background data on sensor storage and stability has been generated, including an investigation of the effect of various interferents in human blood (see Supporting Information). Glucose exhibits a far higher signal than any other analyte present in blood and we expect the sensors to perform similarly to our observations as subcutaneous sensing suffers less from these interferents.[26] We also evaluated the potential biocompatibility of the sensors through a cytotoxicity assay (MTT). Sensors were soaked in PBS for 1, 3, and 7 d and mammalian cells were exposed to the incubated solution. Figure 2 also shows the

DOI: 10.1002/adhm.201300142

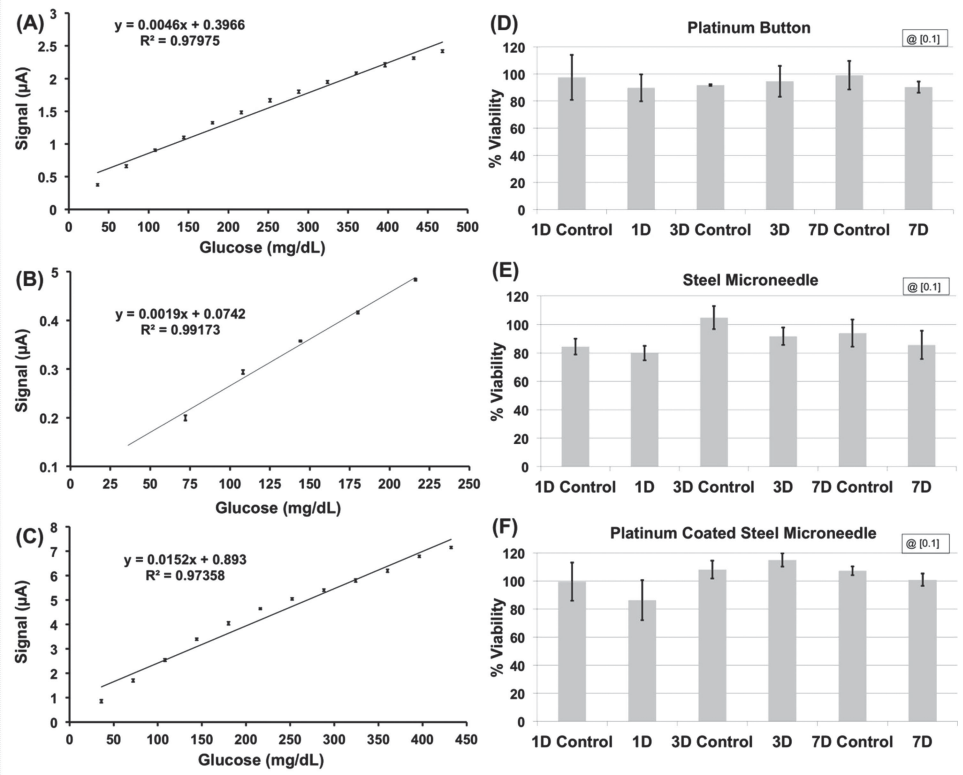

Figure 2. Representative concentration curves for amperometric sensors on A) platinum button, B) steel microneedle, and C) platinum-coated steel microneedle electrodes. Corresponding cytotoxicity data for 1, 3, and 7 d of storage in phosphate-buffered saline for D) platinum button, E) steel microneedle, and F) platinum-coated steel microneedle sensors.

cytotoxicity profiles of these sensors over the course of 1, 3, and 7 d soaked in 5 mL of PBS. No statistically significant changes in cell viability were observed for any of the sensors tested, indicating that toxic components were not excreted from the sensor.

We next investigated their long-term stability of the glucose sensors for their use as part of a patch-based theranostic system. Films were prepared on platinum button controls, steel microneedles, and platinum-coated steel microneedles and subjected to 1, 3, and 7 d of storage at room temperature either wet (PBS) or dry (in an empty parafilmed vial). The performance of the platinum button sensors was not significantly altered by any of these storage conditions. Wet storage resulted in a more reproducible slope of the calibration curve with a deviation of 18% as compared with 67% variability for dry storage. Following 1-d, 3-d, and 7-d dry storage, sensors were linear up to 486 mg dL^{-1} ($y = 0.0079x + 0.2947$; $R^2 = 0.99$, $S/N = 11$), 288 mg dL^{-1} ($y = 0.0047x + 0.1295$; $R^2 = 0.99$, $S/N = 10$), and 360 mg dL^{-1} ($y = 0.0177x + 0.6762$; $R^2 = 0.99$, $S/N = 35$), respectively. Wet-stored platinum buttons showed more similar calibration equations. Following 1-d, 3-d, and 7-d wet storage, sensors were linear up to 216 mg dL^{-1} ($y = 0.0279x + 0.8447$; $R^2 = 0.98$, $S/N = 30$), 288 mg dL^{-1} ($y = 0.0217x + 0.7373$; $R^2 = 0.97$, $S/N = 56$), and 324 mg dL^{-1} ($y = 0.02x + 1.4734$; $R^2 = 0.94$, $S/N = 26$), respectively.

Steel-based sensors demonstrated relatively poor performance, exhibiting low sensor ranges and, in the case of 7-d wet, essentially ceased to function entirely (**Figure 3**E). Sensor responses for 1-d, 3-d, and 7-d dry storage were up to 144 mg dL^{-1} each ($y = 0.0033x + 0.1077$; $R^2 = 0.99$, $S/N = 30$), ($y = 0.0014x + 0.0425$; $R^2 = 0.99$, $S/N = 9$), and ($y = 0.0029x - 0.0068$; $R^2 = 0.99$, $S/N = 23$), respectively. 1-d, 3-d, and 7-d wet sensors showed linearity to 216 mg dL^{-1} ($y = 0.0332x - 0.0302$; $R^2 = 0.99$, $S/N = 39$), 180 mg dL^{-1} ($y = 0.0515x + 1.1087$; $R^2 = 0.98$, $S/N = 24$), and 216 mg dL^{-1} ($y = 0.001x + 0.0213$; $R^2 = 0.99$, $S/N = 13$), respectively. The signal for the 7-d wet sensor, however, was an order of magnitude lower than that for the other sensors.

Finally, both wet and dry storage yielded very similar calibration trends for the platinum-coated steel microneedles, with high linearity and S/N ratios. In the case of dry sensors, it was more typical for the 7-d systems to have an extended sensing ratio. 1-d and 3-d dry sensors had ranges up to 324 mg dL^{-1} ($y = 0.0186x + 0.832$; $R^2 = 0.98$, $S/N = 13$) and ($y = 0.0157x + 0.9182$; $R^2 = 0.97$, $S/N = 12$), respectively, while the 7-d sensor made it to 396 mg dL^{-1} ($y = 0.0162x + 1.1239$; $R^2 = 0.97$, $S/N = 18$). Platinum-coated steel microneedles stored wet had shortened ranges with the same linearity, however, 252 mg dL^{-1} ($y = 0.007x + 0.1707$; $R^2 = 0.98$, $S/N = 5$), 216 mg dL^{-1} ($y = 0.0073x - 0.0659$; $R^2 = 0.99$, $S/N = 8$), and 252 mg dL^{-1} ($y = 0.007x + 0.1305$; $R^2 = 0.99$, $S/N = 23$), respectively. These results are encouraging for the use of the microneedle sensor in vivo over the course of the "Smart Patch" lifetime.

The microneedle patch holds the potential for a noninvasive, non-encumbering platform for both sensing and delivery.

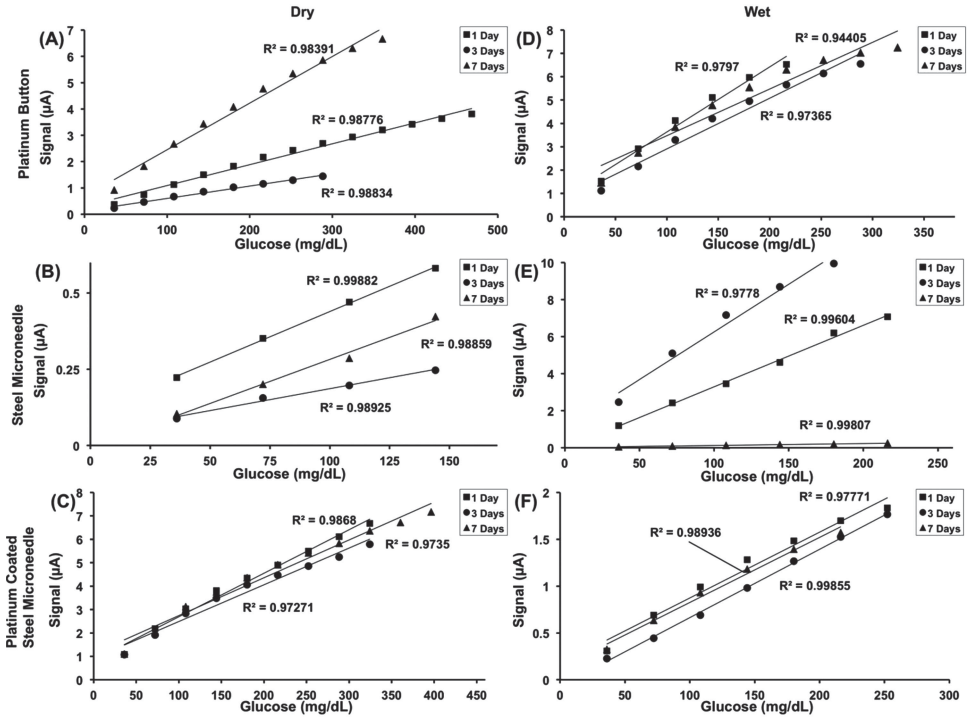

Figure 3. Long-term storage of prepared sensors showing dry (left) and wet (right) storage conditions. A) and D) are platinum button electrodes, B) and E) are steel electrodes, and C) and F) are platinum-coated steel electrodes.

Multiple sensor arrays may be used to address the issue of redundancy (for signal averaging as well as combating single-sensor failure). The patch could be easily applied and removed, aiding patient compliance with blood glucose monitoring, a major concern for many diabetics. Our data indicate that these sensors are linear and reliable throughout the physiological range and are nontoxic. The concept of a transcutaneous electrode array that can accomplish numerous functions for diabetes theranostics is an attractive solution to the treatment of this disease. The sensors investigated in this study represent one potential component of such a "smart patch."

Experimental Section

Sensor Preparation: The polymerization bath was prepared by adding 13.3 mg of *p*-toluenesulfonic acid (PTSA; Sigma–Aldrich) to 100 mL of deionized water. The pH of the bath was 3.4. For initial platinum button controls, fresh polymerization solution was prepared by adding 50 mg of GOx from *Aspergillus niger* (Sigma–Aldrich) to 5 mL of polymerization. The vial was then shaken to mix. Next, 15 μL of 3,4-ethylenedioxythiophene (EDOT; Aldrich) was added to the solution. The solution was then stirred with a magnetic stir bar at 600 rpm for 1 h. Fresh polymerization solution was prepared on each day of experiments. Polymerization was carried out at 1.5 V for 15 s. The platinum electrodes (CH Instruments) were polished using micropolish powder (0.05 μm; CH Instruments) between uses.

Stainless steel (316L) 2D arrays (4.901 mm across × 5.693 mm tall × 127 μm thick) of microneedles were purchased from eMachineShop. The microneedles are 680 μm in length and 250 μm wide. Needles were used as-is and coated with platinum. Platinum was deposited using sputter coating (using an AJA Orion 5 Sputterer) using a 50-nm titanium base and an overcoat of 450 nm of platinum metal.

For both types of microneedle arrays, fresh polymerization solution was prepared by adding 50 mg of GOx from *A. niger* (SIGMA) to 4 mL of polymerization bath. The vial was then shaken to mix. 15 μL of EDOT was then added to 1 mL of acetonitrile (Sigma–Aldrich). These two solutions were then mixed. The solution was then shaken and stirred with a magnetic stir bar at 600 rpm for 1 h. Fresh polymerization solution was prepared on each day of experiments. Polymerization of the sensor onto steel microneedles was carried out in the same manner as the platinum buttons excepting the polymerization duration was 30 s.

Steel microneedles were initially cleaned by sonication in acetone for 1 min. Teflon tape (PTFE Thread Sealant Tape) was used to cover the microneedle platform and solely expose the microneedle portion. The polymerization and sensing protocols used were identical to the ones used for platinum buttons. Platinum-coated microneedles were tested with the same protocol (15 s polymerization duration).

Sensor Testing: The platinum buttons were dried in air for 1 h prior to sensing. A 2.0 M solution of glucose (Acros Organics) was prepared in PBS (calcium chloride, magnesium chloride, pH 7.4, 1X; GIBCO life technologies). This was used to make sequential additions to the sensing bath. 10 mL of PBS was added to the electrochemical cell. The electrode (sensor), a counter electrode flag (platinum), and a reference electrode (silver) were submerged together to form the cell. A magnetic stir bar was added and allowed to stir at 300 rpm.

Chronoamperometry was used to measure the change in current with time over the course of glucose additions to the stirred bath (300 rpm). The potentiostat was initially allowed to stabilize to a steady horizontal current value prior to sensor calibration. 10 μL of the 2.0 M glucose solution (corresponding to a 2×10^{-3} M final concentration of glucose, or 36 mg dL^{-1}) was added to the vial and the signal was allowed to stabilize. The stable current was then recorded. Addition of 10 μL aliquots of glucose was added, stabilized, and recorded up to a maximum of 40×10^{-3} M. For long-term stability tests, the sensors were stored either dry in air or in PBS for 1, 3, and 7 d prior to sensing.

Cytotoxicity Assay: Platinum button and both microneedle-type sensors were used to prepare sensors, which were then submerged

DOI: 10.1002/adhm.201300142

in 5 mL of PBS for 1, 3, and 7 d. The PBS was used to carry out a biocompatibility screen (cytotoxicity) using an MTT assay. CHO (ATCC) cells were seeded into 96 well plates at 2500 cells/well and allowed to grow overnight before treatment. After 48 h exposure, cells were given MTT (20 µL, 5 mg mL^{-1}) and incubated for 4 h. Cells were subsequently lysed with 100 µL DMSO and viability was measured with a plate reader (Tecan M200Pro) at 565 nm. Serial dilutions of the stock solution in which the sensors were stored were carried out to give a wide range of possible concentrations for comparison (0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001, and 0.0000001).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work was supported by grant 09PG-T1D027 from the Leona M. and Harry B. Helmsley Charitable Trust, a postdoctoral fellowship from the Juvenile Diabetes Research Foundation (BCT), and a generous gift from the Tayebati Family Foundation.

Received: April 18, 2013
Revised: June 20, 2013
Published online:

[1] World Health Organization, WHO | Diabetes, http://www.who.int/mediacentre/factsheets/fs312/en/, accessed: August 2012.
[2] American Diabetes Association, Diabetes Statistics 2012, http://www.diabetes.org/diabetes-basics/diabetes-statistics/?loc = DropDownDB-stats, accessed: August 2012.
[3] B. C. Gilligan, M. Shults, R. K. Rhodes, P. G. Jacobs, J. H. Brauker, T. J. Pintar, S. J. Updike, *Diabetes Technol. Ther.* **2004**, *6*, 378.
[4] S. Martin, B. Schneider, L. Heinemann, V. Lodwig, H.-J. Kurth, H. Kolb, W. A. Scherbaum, *Diabetelogia* **2006**, *49*, 271.
[5] K. Bratlie, R. L. York, M. A. Invernale, R. S. Langer, D. G. Anderson, *Adv. Healthcare Mater.* **2012**, *1*, 267.
[6] http://www.dexcom.com/seven-plus, accessed: August 2012; http://www.freestylenavigator.com; Abbott, accessed: August 2012, http://www.medtronicdiabetes.net/Products/ParadigmRevelPump, accessed: August 2012.
[7] N. Mauras, R. Beck, D. Xing, K. Ruedy, B. Buckingham, M. Tansey, N. H. White, S. A. Weinzimer, W. Tamborlane, C. Kollman, *Diabetes Care* **2012**, *35*, 204.
[8] S. Swain, S. Beg, A. Singh, C. N. Patro, M. E. B. Rao, *Curr. Drug Delivery* **2011**, *8*, 456.
[9] S. H. Bariya, M. C. Gohel, T. A. Mehta, O. P. Sharma, *J. Pharm. Pharmacol.* **2012**, *64*, 11.
[10] J. D. Zahn, Y.-C. Hsieh, M. Yang, *Diabetes Technol. Ther.* **2005**, *7*, 536.
[11] W. Martanto, S. P. Davis, N. R. Holiday, J. Wang, H. S. Gill, M. R. Prausnitz, *Pharm. Res.* **2004**, *21*, 947.
[12] B. P. Chaudhri, F. Ceyssens, P. De Moor, C. Van Hoof, R. Puers, *J. Micromech. Microeng.* **2010**, *20*, 064006.
[13] D. V. McAllister, P. M. Wang, S. P. Davis, J.-H. Park, P. J. Canatella, M. G. Allen, M. R. Prausnitz, *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13755.
[14] J. C. Harper, S. M. Brozik, J. H. Flemming, J. L. McClain, R. Polsky, D. Raj, G. A. T. Eyck, D. R. Wheeler, K. E. Achyuthan, *ACS Appl. Mater. Interfaces* **2009**, *1*, 1591.
[15] C. Tautorat, P. J. Koester, J. Held, J. Gaspar, P. Ruther, O. Paul, A. Cismak, A. Heilmann, J. Gimsa, H. Beikirch, L. Jonas, W. Baumann, *12th Int. Conf. Miniaturized Systems for Chemistry and Life Sciences* **2008**, *12*, 1777.
[16] N.-F. Chiu, J.-M. Wang, C.-W. Liao, C.-H. Chen, H.-C. Chen, L.-J. Yang, S.-S. Lu, C.-W. Lin, *Proc. of the 2005 IEEE Engineering in Medicine and Biology 27th Annual Conf*, Shanghai, China, **2005**.
[17] P. R. Miller, S. A. Skoog, T. L. Edwards, D. M. Lopez, D. R. Wheeler, D. C. Arango, X. Xiao, S. M. Brozik, J. Wang, R. Polsky, R. J. Narayan, *Talanta* **2012**, *88*, 739.
[18] I. Park, Z. Li, X. Li, A. P. Pisano, R. S. Williams, *Biosens. Bioelectron.* **2007**, *22*, 2065.
[19] P. R. Miller, S. D. Gittard, T. L. Edwards, D. M. Lopez, X. Xiao, D. R. Wheeler, N. A. Monteiro-Riviere, S. M. Brozik, R. Polsky, R. J. Narayan, *Biomicrofluidics* **2011**, *5*, 13415.
[20] S. Zimmermann, D. Fienbork, B. Stoeber, W. Flounders, D. Liepmann, *12th Int. Conf. on Solid State Sensors, Actuators and Microsystems* **2003**, *12*, 99.
[21] J. R. Windmiller, N. Zhou, M.-C. Chuang, G. Valdés-Ramírez, P. Santhosh, P. R. Miller, R. Narayan, J. Wang, *Analyst* **2011**, *136*, 1846.
[22] S.-O. Choi, Y. C. Kim, J.-H. Park, J. Hutcheson, H. S. Gill, Y.-K. Yoon, M. R. Prausnitz, M. G. Allen, *Biomed. Microdevices* **2010**, *12*, 263.
[23] J. D. Nelson, M. A. Woelk, S. Sheps, *Diabetes Care* **1983**, *6*, 262.
[24] A. Heller, B. Feldman, *Chem. Rev.* **2008**, *108*, 2482.
[25] S. Alva, *J. Diabetes Sci. Technol.* **2008**, *2*, 546.
[26] H. Wiig, M. A. Swartz, *Physiol. Rev.* **2012**, *92*, 1005.