INKJET PRINTING OF FUNCTIONALIZED SILK PROTEINS FOR ENHANCED STABILITY AND COLORIMETRIC BACTERIAL SENSING APPLICATIONS

H. Tao[1], B. Marelli[2], M. Yang[2], B. An[2], D.L. Kaplan[2] and F.G. Omenetto[2]
[1]State Key Lab of Transducer Technology, Shanghai Institute of Microsystem and Information Technology, CAS, Shanghai, China
[2]Department of Biomedical Engineering, Tufts University, Medford, USA

ABSTRACT

We present the use of functionalized silk proteins for enhanced stability of biochemically active agents (e.g. horseradish peroxidase) in printed silk materials without the need of refrigeration storage. Direct printing of polydiacetylene vesicles conjugated with goat IgG antibody in printed silk materials for colorimetric detection of *E. coli*. has also been demonstrated.

KEYWORDS

Silk, stabilization, printing, colorimetric sensing

INTRODUCTION

The development of new technologies has combined progress in fabrication techniques with new discoveries in material science. Printing technologies leverage on robust, economic and user-friendly integrated systems and have been actively employed at various occasions [1-5]. Efforts have been largely focused on developing non-toxic, green materials to be coupled with printing technologies.

Silk - known and used as a high quality textile material for thousands of years - has extended its splendor to the field of biomedical engineering, as a "versatile" biomaterial, for its remarkable biocompatibility and unique mechanical and optical properties [6-12].

As a shift in the current scenario, we sought to develop functional inks for printing where functions come from the hybridization of silk proteins with biochemically active molecules as sensory materials for the detection of biologically-, environmentally- and chemically-important target molecules.

METHOD

Preparation and usage of those silk proteins do not involve with the use of any hostile organic or inorganic solvents but only water and desired bioactive compounds and are compatible with commercial inkjet printers. We chose drop-on-demand, piezoelectric-based inkjet printing as a versatile method for precise material deposition (Dimatix DMP 2800, FUJIFILM) [Figure 1].

Ink cartridges were cleaned/flushed with deionized water before use. All liquids (i.e. silk inks and controls) were filtered through a nylon membrane with a 0.2 µm pore size. A drop watcher is equipped, which allows real-time observation and capture of drop formation on the nozzles and printing trajectory of the drops after ejection. A full set of parameters (e.g. waveform, pulse width, individual nozzle voltage, firing frequency, cartridge and substrate temperatures, cleaning cycles) are accessible to optimize for various inks [13].

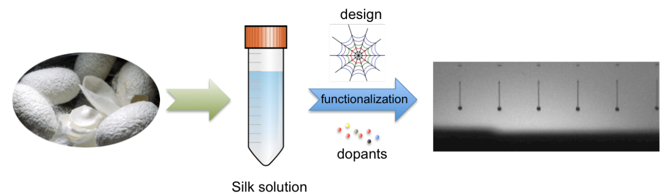

Figure 1: Silk proteins are extracted from naturally silk sources (e.g. silk cocoons, silk webs and raw silk fibers) and purified through a water-dialysis process, resulting in an aqueous solution of silk proteins that can be further functionalized by doping with bioactive agents.

The silk protein inks in this work were prepared by first degumming (sericin removal) of *Bombyx mori* silk followed by a water-based extraction and purification process. We prepared two sets of functionalized silk proteins "inks" doped with horseradish peroxidase (HRP) and polydiacetylenes (PDAs) vesicles conjugated with goat IgG antibody, respectively, for enhanced stability and colorimetric bacterial sensing demonstrations. No refrigeration was used.

Enzymatic activity of HRP-doped silk solution before and after printing was evaluated and compared by a colorimetric ELISA for horseradish peroxidase/ 3, 3', 5, 5'-Tetramethylbenzidine (HRP/TMB). The HRP activity was measured by adding 100 µL of TMB in 96 well plates containing 10 µL of HRP-containing solution for 1 min at room temperature and the reaction was stopped by the addition of 100 µL 0.1 mol/L sulfuric acid. Absorbance was measured at 450 nm using a commercial 96 well plate reader (BMG Labtech Inc., Durham, NC).

Lyophilized E. coli cultures was reconstituted and expanded according to instructions provided by American Type Culture Collection (ATCC). To test susceptibility, bacteria cultures were grown in liquid Tryptic Soy Broth for 18–24 h to an optical density (OD@600) between 0.8 and 1 (corresponding to a viable count of approx. 10^7 – 10^8 CFU/mL).

PDA vesicles were obtained as previously reported [14, 15] and conjugated with goat IgG through an HRP mediated reaction, which was stopped by an excess of H_2O_2 and copper (30 µM). DA-IgG were then blended in silk-based inks and printed on transparent plastic substrates and surgical gloves.

RESULT AND DISCUSSION

Enzymes are widely used to develop functional materials for many applications, which however are often hampered by a lack of long-term stability under processing conditions. Stabilization of enzymes has been demonstrated using silk biomaterials (particularly in bulk-loaded films and scaffolds) for medical, diagnostic and biosensing applications [16, 17]. In this work, we used HRP (Type VI, Sigma; 250 – 333 unit/mg), a widely used enzyme as an indicator in immunoassays but lacks stability in solution, to test the effectiveness of stabilization of enzyme activities using silk protein inks. Enzymatic activities of HRP doped silk inks were evaluated by a colorimetric ELISA for HRP using the solution of TMB [Figure 2].

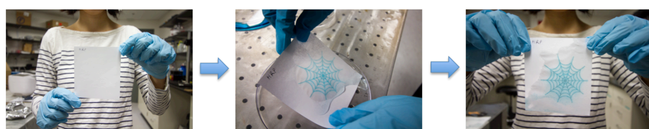

Figure 2: An example of utilization of functionalized silk proteins for an enzyme linked immunospecific assay (ELISA) test was given, showing that HRP doped silk proteins printed on paper changed the color (from colorless) to blue after being exposed to the solution of TMB.

For comparison, same patterns were also printed using HRP only solution. Both sets of samples (N=3) were stored and were checked continuously at certain time points. HRP activities in silk inks were found considerably higher at all circumstances, underscoring the enhanced stability due to silk proteins [Figure 3].

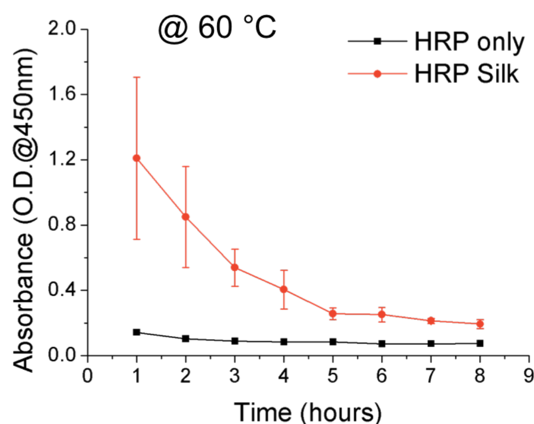

Figure 3: HRP activities in silk inks were found considerably higher than HRP only solution at the elevated temperature of ~ 60°C.

Similar stabilization of HRP activities during inkjet printing was observed previously though several additives such as glycerol, Triton, and sodium carboxymethyl cellulose were needed to adjust the rheological properties and to reduce the evaporation of inks [18]. One true power of as reported silk inks lies in the long-term stabilization after printing at ambient and adverse conditions (for example, at high temperatures), which eliminates the need of "cold chain" [19].

Owing to the ease with which silk inks can be prepared in a water-based process, a wide set of opportunities can be foreseen by combining silk proteins with other macromolecules such as antibodies and functional polymers. For demonstration, we designed a colorimetric bacterial sensor that was made by directly printing functionalized silk inks on surgical gloves and immobilizing/stabilizing PDA vesicles conjugated with goat IgG antibody in printed silk materials for detection of *E. coli*. PDA vesicles were conjugated with goat IgG through an HRP mediated reaction. PDA-IgG vesicles were then blended in silk-based inks and printed, to exert the stabilization function of silk over the labile antibody molecule.

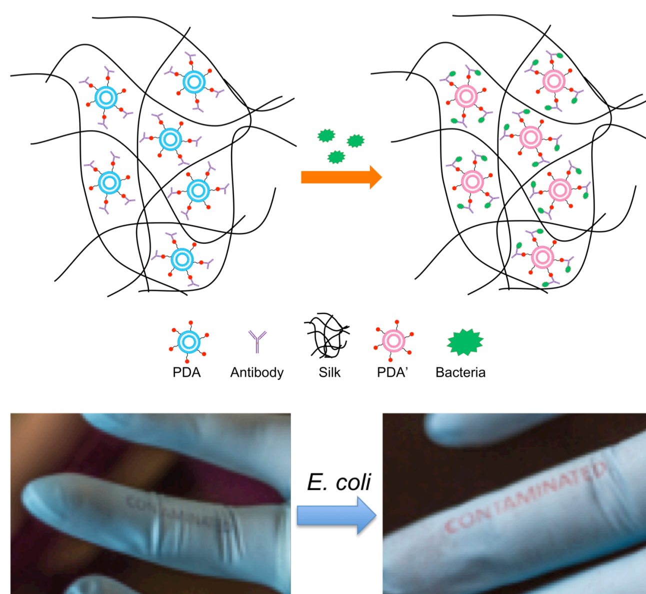

Figure 4: A colorimetric bacterial sensor for the detection of E. coli by printing polydiacetylenes (PDA) and goat IgG antibody immobilized in silk matrix on surgical gloves.

The sensor was then based on the intrinsic color change of PDA from blue to red (showing "CONTAMINATED", produced by alteration (i.e. stretching) of PDA conformation under external stimuli (e.g. exposure to *E. coli* bacteria with a concentration of ~ 10^4 CFU/mL) [Figure 4]. This type of sensors would be particularly attractive for in situ assessment and diagnosis of pathogenic contamination and resistant infection where sensors directly interface with contamination sources, including the body, food, and clinical equipment.

CONCLUSION

In summary, we demonstrate the viability of direct printing and stabilization of labile bioactive compounds using silk proteins as a versatile bioprinting approach for enhanced stability and colorimetric sensing applications.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge support from NSF-INSPIRE Grant (DMR-1242240), and from the NIH P41 (EB002520). H.T. and F.G.O. would like to thank the Science and Technology Commission of Shanghai Municipality for the support under the International Collaboration Project (14520720400).

REFERENCES

[1] C. Kucukgul, S. B. Ozler, I. Inci, E. Karakas, S. Irmak, D. Gozuacik, *et al.*, "3D Bioprinting of Biomimetic Aortic Vascular Constructs with Self-Supporting Cells," *Biotechnol Bioeng,* Nov 11 2014.

[2] S. Wust, R. Muller, and S. Hofmann, "3D Bioprinting of complex channels-Effects of material, orientation, geometry, and cell embedding," *J Biomed Mater Res A,* Dec 18 2014.

[3] S. V. Murphy and A. Atala, "3D bioprinting of tissues and organs," *Nat Biotechnol,* vol. 32, pp. 773-85, Aug 2014.

[4] S. Tasoglu and U. Demirci, "Bioprinting for stem cell research," *Trends in Biotechnology,* vol. 31, pp. 10-19, Jan 2013.

[5] B. Derby, "Printing and Prototyping of Tissues and Scaffolds," *Science,* vol. 338, pp. 921-926, Nov 16 2012.

[6] D. N. Rockwood, R. C. Preda, T. Yucel, X. Q. Wang, M. L. Lovett, and D. L. Kaplan, "Materials fabrication from Bombyx mori silk fibroin," *Nature Protocols,* vol. 6, pp. 1612-1631, Oct 2011.

[7] C. Vepari and D. L. Kaplan, "Silk as a biomaterial," *Progress in Polymer Science,* vol. 32, pp. 991-1007, Aug-Sep 2007.

[8] E. M. Pritchard and D. L. Kaplan, "Silk fibroin biomaterials for controlled release drug delivery," *Expert Opinion on Drug Delivery,* vol. 8, pp. 797-811, Jun 2011.

[9] A. Matsumoto, A. Lindsay, B. Abedian, and D. L. Kaplan, "Silk Fibroin Solution Properties Related to Assembly and Structure," *Macromolecular Bioscience,* vol. 8, pp. 1006-1018, Nov 10 2008.

[10] G. H. Altman, F. Diaz, C. Jakuba, T. Calabro, R. L. Horan, J. S. Chen, *et al.*, "Silk-based biomaterials," *Biomaterials,* vol. 24, pp. 401-416, Feb 2003.

[11] Q. Lu, X. Hu, X. Q. Wang, J. A. Kluge, S. Z. Lu, P. Cebe, *et al.*, "Water-insoluble silk films with silk I structure," *Acta Biomaterialia,* vol. 6, pp. 1380-1387, Apr 2010.

[12] H. J. Jin, J. Park, V. Karageorgiou, U. J. Kim, R. Valluzzi, and D. L. Kaplan, "Water-stable silk films with reduced beta-sheet content," *Advanced Functional Materials,* vol. 15, pp. 1241-1247, Aug 2005.

[13] Y. F. Liu, M. H. Tsai, Y. F. Pai, and W. S. Hwang, "Control of droplet formation by operating waveform for inks with various viscosities in piezoelectric inkjet printing," *Applied Physics a-Materials Science & Processing,* vol. 111, pp. 509-516, May 2013.

[14] Y. J. Song, W. L. Wei, and X. G. Qu, "Colorimetric Biosensing Using Smart Materials," *Advanced Materials,* vol. 23, pp. 4215-4236, Oct 4 2011.

[15] Y. K. Jung, T. W. Kim, J. Kim, J. M. Kim, and H. G. Park, "Universal colorimetric detection of nucleic acids based on polydiacetylene (PDA) liposomes," *Advanced Functional Materials,* vol. 18, pp. 701-708, Mar 11 2008.

[16] Q. Lu, X. Q. Wang, X. Hu, P. Cebe, F. Omenetto, and D. L. Kaplan, "Stabilization and Release of Enzymes from Silk Films," *Macromolecular Bioscience,* vol. 10, pp. 359-368, Apr 8 2010.

[17] S. Z. Lu, X. Q. Wang, Q. Lu, X. Hu, N. Uppal, F. G. Omenetto, *et al.*, "Stabilization of Enzymes in Silk Films," *Biomacromolecules,* vol. 10, pp. 1032-1042, May 2009.

[18] A. Fishman, I. Levy, U. Cogan, and O. Shoseyov, "Stabilization of horseradish peroxidase in aqueous-organic media by immobilization onto cellulose using a cellulose-binding-domain," *Journal of Molecular Catalysis B-Enzymatic,* vol. 18, pp. 121-131, Sep 13 2002.

[19] J. E. Zhang, E. Pritchard, X. Hu, T. Valentin, B. Panilaitis, F. G. Omenetto, *et al.*, "Stabilization of vaccines and antibiotics in silk and eliminating the cold chain," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 109, pp. 11981-11986, Jul 24 2012.

CONTACT

*F.G. Omenetto, tel: +1-617 627 4972; fiorenzo.omenetto@tufts.edu; H. Tao, B. Marelli, M. Yang contributed equally to this work.