BIOMIMETIC BARBED MICRONEEDLES FOR HIGHLY ROBUST TISSUE ANCHORING

Sanwei Liu[1,2,4], Sangwook Chu[1,2,4], George E. Banis[2,3,4], Luke A. Beardslee[2], and Reza Ghodssi[1,2,3,4]
[1]Department of Electrical and Computer Engineering, [2]Institute for Systems Research,
[3]Fischell Department of Bioengineering, [4]Robert E. Fischell Institute for Biomedical Devices,
University of Maryland, College Park, Maryland, USA

ABSTRACT

We present a bioinspired tissue-anchoring technology enabled via direct laser writing (DLW). 3-D printed barbed microneedles, mimicking the parasitic spiny-headed worm, display excellent structural fidelity/resolution and demonstrate -0.6 mN penetration force and 25 mN pull-out force when characterized on porcine small intestine tissue. Compared to the state-of-the-art barbed microneedles, the results indicate a significant advancement with approximately two orders of magnitude lower penetration force and over ten-fold higher pull-out/penetration ratio (PPR). The ease of tissue penetration and strength of attachment characteristics allow a more passive anchoring mechanism, with lower actuation and power requirements, for use in minimally invasive gastrointestinal (GI) resident devices.

KEYWORDS

Barbed Microneedle, Biomimetics, Gastrointestinal Tract, Tissue Anchoring, Direct Laser Writing

INTRODUCTION

Barbed needles are naturally occurring tissue anchoring architectures found on porcupine quills, mosquito and honeybee stingers, as well as parasite proboscis [1]–[4]. Recent studies investigating barbed microneedles have demonstrated that the microbarbs along the needle surface enhance mechanical anchoring. Notably, they provide an increased pull-out force during removal as compared to the force required for penetration into skin and muscle tissues [1], [5]. This biological architecture has significant potential for anchoring sensors and drug delivery devices within tissue with reduced active control and preloading [6], especially within the hard-to-access regions of the human gastrointestinal (GI) tract mucosa that provides a rich amount of sensing and therapeutic opportunities [7], [8].

However, the development of bioinspired barbed microneedles has been limited by either fabrication methods or materials that are incapable of precisely replicating biological models, ultimately inhibiting performance. Using silicon-based material, early development of micro-spikes demonstrated effective mechanical fastening on human skin with anchoring strength of 3.4 kPa (or 21 mN per needle) [8], which would potentially be able to resist removal from GI tissue via peristalsis force (~1 kPa) [9]–[11]. However, the structure is stiff enough (with Young's modulus over 100 GPa) to potentially cause perforation, and fabricable features are limited by the conventional micromachining capabilities [12]. Using softer polymers, Yang et al. developed a softer two-layer polymer microneedle with a swellable tip, mimicking a shape-changing parasitic spiny-head worm proboscis. The demonstrated tissue anchoring strength is 4.5 kPa (or 45 mN per needle) on porcine small intestine tissue [13]. However, penetration requires preloading (~10 kPa) that is more than two times higher than the pull-out to ensure robust tissue-anchoring, which would be undesirable for GI application. Recent bio-inspired barbed microneedles, such as molded North American porcupine quills and magnetorheological drawing worker honeybee stingers, have achieved tissue anchoring with low penetration force and higher pull-out force [1], [5]. However, the demonstrated PPR (~1.8) are much lower than their biological models (~20), respectively [3].

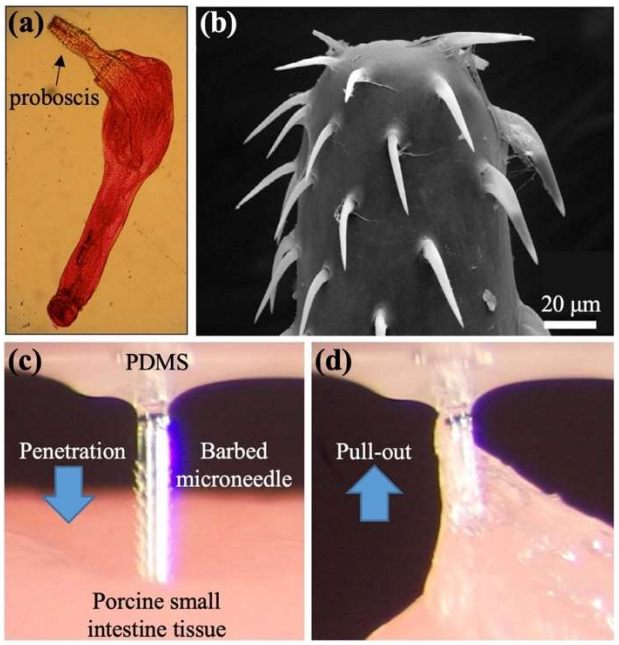

Figure 1: (a) Optical image of a spiny headed worm (courtesy of N. Campbell, Wikipedia), and (b) SEM image of the anterior end of a worm proboscis [14]. *(c-d) optical images showing a barbed microneedle (c) penetrating and (d) pulling out of a porcine small intestinal tissue sample with tissue attachment on microbarbs.*

In this work, we present a 3-D printed biomimetic barbed microneedle with high fidelity/resolution, attaining significantly reduced preloading as well as strong tissue anchoring capabilities. The parasitic, spiny-headed worm (Figure 1a), which is the biological model for this work, uses backward-pointing microbarbs on its microneedle-sized proboscis (Figure 1b) that allow it to evaginate through the mucosa and fasten to the gut wall with minimal locomotion [4]. For the first time, a microscale replication of the spiny proboscis has been achieved, demonstrating highly efficient tissue anchoring on porcine small intestinal

tissues (Figure 1c, 1d). The utilization of this technology will ultimately aid in the development of ingestible capsule systems, particularly for deploying GI resident devices [7] to embed within the intestinal epithelium for prolonged monitoring and drug release.

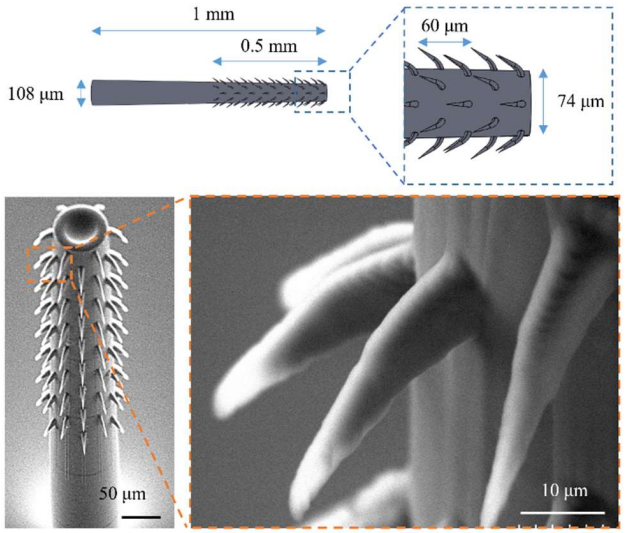

Figure 2: Schematic with dimensions (top) and scanning electron microscope (SEM) images (bottom) of a barbed microneedle fabricated via 3-D DLW. The zoomed-in image depicts the high-fidelity fabrication of the curved barbs with sharp tips (~ 1 µm resolution).

Microneedle design

Taking advantage of the DLW technology, which utilizes two-photon photopolymerization with submicron resolution, the 3-D printed barbed microneedles display high precision biomimicking structures as shown in Figure 2. The design of the surface microbarbs and needle trunk are based on the spiny-headed worm morphology [14], as observed with SEM image in Figure 1b, with minor modification (i.e. hollow needle and uniform microbarb design) for DLW fabrication feasibility. The needle diameter decreases from 108 µm at the base to 74 µm at the tip, mimicking the truncated geometry of the worm proboscis in Figure 1b. The needle diameter in this work is nearly 50% narrower than the worker honeybee stinger (~200 µm) to enhance penetration and reduce perforation [5]. The 1 mm needle height was designed to increase the probability of penetrating the gut wall deep enough to pass the mucus blanket and epithelial barrier [15], [16]. The needle is designed to be hollow with 15 µm wall thickness to reduce over 50% of the laser writing time and increase flexibility to potentially help avoid the risk of fracture while in tissue.

A total of 96 microbarbs are arranged in a quincuncial pattern resembling the dense formation of the worm proboscis [4]. The blunt needle design allows a maximum of six microbarbs to be evenly arranged around the circumference, with base diameter, curvature length and angle of 8 µm, 40 µm to 30 µm (tip to bottom), and ~50°, respectively. The microbarbs cover the first 0.5 mm of needle anterior length at a 60 µm axial spacing, which is the most effective region for tissue anchoring according to the study of barbed porcupine quills [1]. Additionally, the microneedle material has a Young's modulus of about 4-5 GPa, which is much softer than silicon, ensuring safe tissue penetration [12], [17].

EXPERIMENTAL METHODS

Two-photon DLW and needle assembly

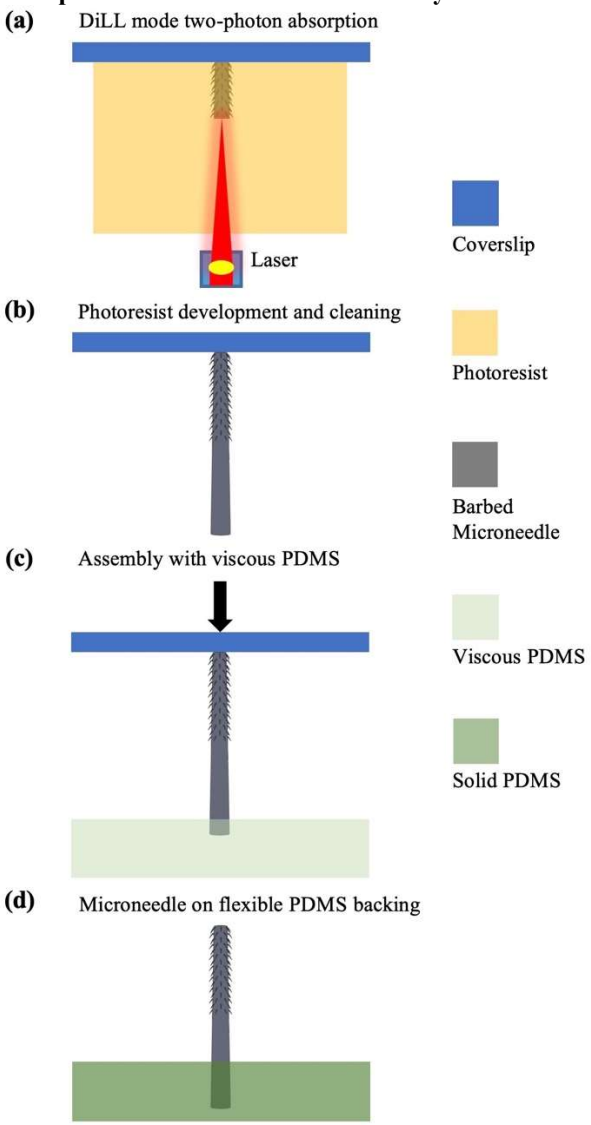

Figure 3: Fabrication and assembly process. (a) two-photon absorption with 25X objective lens (NA = 0.8, DiLL-mode) and negative-tone photoresist (IP-S) at 80 mW laser power, 40 mm/s scan speed, 1 µm slicing, and 500 nm hatching distances. The writing block X-Y-Z dimensions are 250 µm, 250 µm, and 150 µm respectively. (b) 20-min photoresist development in propylene glycol monomethyl ether acetate (PGMEA) followed by a 2-min isopropyl alcohol (IPA) cleaning. (c) Microneedle assembly via dipping the needle base in 10:1 PDMS mixture (Sylgard 184, Dow Corning, Corning, NY) for 4 minutes at 100℃, leading to the final (d) microneedle on a 1 mm thick flexible backing.

Figure 3 depicts the barbed microneedle fabrication flow developed in this work, including the DLW fabrication and an assembly process to attach the microneedle to a flexible PDMS backing. Using the Photonic Professional GT system (Nanoscribe GmbH,

Karlsruhe, Germany), the CAD design is fabricated using the Dip-in Laser Lithography (DiLL) mode with an ~8 minute processing time. Figure 3a presents the two-photon absorption process, creating the microneedle starting from needle tip instead of the base. This inverse writing orientation is ideal for obtaining the precise geometric curvature (angle and sharpness) of the overhanging barbs as it allows printing of a contiguous structure without free-floating material at any step.

Figure 3b shows the fabricated structure after photoresist development and cleaning. In order to overcome the weak interface adhesion between the needle and the coverslip and to facilitate tissue anchoring applications, the inversely oriented microneedle is attached to a flexible PDMS backing substrate (Figure 3c-d). Benefiting from the inverse writing orientation in which the needle base is exposed in air, the microneedle base can be inserted 0.25 mm into a PDMS layer using a custom aluminum mold heated to 100ºC using a hotplate. Once the PDMS is cured and cooled, the microneedle base is firmly attached to the solid PDMS substrate and can be easily peeled off from the coverslip.

Tissue anchoring mechanical test

The microneedle tissue anchoring performance is characterized by the force and displacement experienced by the needle after undergoing a three-phase motion, including tissue penetration, linear shift, and pull-out. During testing, a mechanical tester (Model 5565, Instron, MA, USA) is used with tensile and compressive modes containing a ±50 N load cell. Thawed porcine small intestinal tissue (Animal Biotech Industries Inc, PA) is cut into an approximately 1 cm × 1 cm patch, and the non-mucosa side is adhered to a 3-D printed fixture. The tissue sample is then placed on the base fixture of the tensile tester, and the microneedle on PDMS substrate is attached to the upper movable sensing column. During the test, the upper column travels downward to insert the microneedle into the tissue mucosa at a rate of 0.01 mm/s until the displacement reaches 0.4 mm from initial needle/tissue contact and holds the penetration for 30 s. After a 1 mm lateral shifting (consistent for test) perpendicular to the penetration - mimicking the peristaltic shear interaction in the small intestine - the microneedle is moved upward (away from the tissue sample) at a rate of 0.01 mm/s until the microneedle has pulled out of the tissue completely.

EXPERIMENTAL RESULTS

Figure 4 presents both the force and displacement of the microneedle measured throughout penetration and pull-out testing on the porcine small intestine, showing a low penetration force of -2 mN, high peak pull-out force of ~40 mN, and PPR of ~20. Figure 5 shows that the average penetration and pull-out forces are measured as -0.6 mN and 25 mN, respectively. Additionally, the average penetration force and pull-out force acquired from barbless microneedles (bare needle without microbarbs) are only -0.3 mN and 1.7 mN, respectively. The significant difference between the barbed and barbless needles strongly indicates that the barbed features play an integral role in increasing the pull-out force, while maintaining a low penetration force.

The data obtained has large standard deviations of 0.7 mN and 33 mN for penetration and pull-out force, respectively, which is partly due to system noise and more importantly, the number of microhooks that become attached to the tissue sample. However, these factors are not critical as more than 60% of the samples demonstrated a PPR over 20 ($P < 0.05$), suggesting significantly enhanced tissue anchoring efficacy.

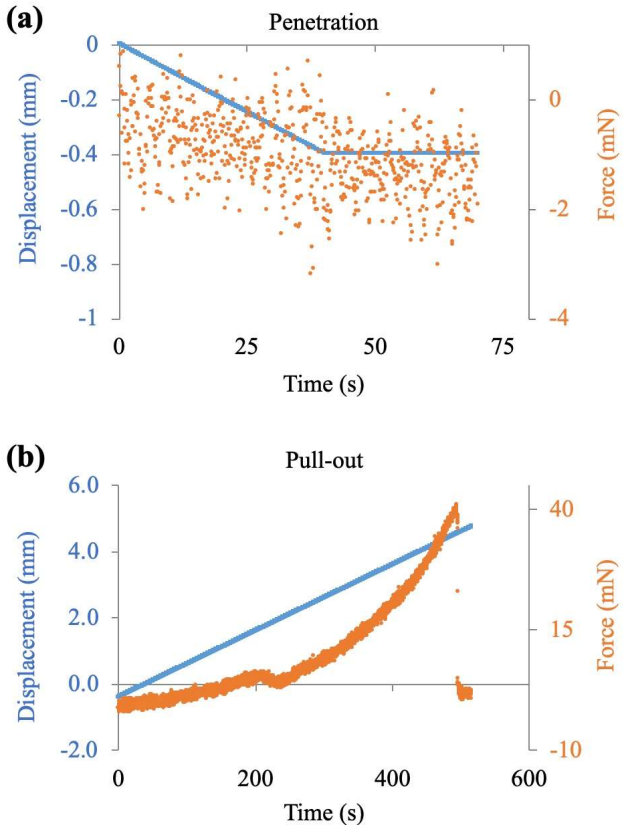

Figure 4: Plots of force and displacement vs. time of needle-tissue interaction during (a) 0.4 mm penetration followed by 30s relaxation, and (b) pull-out following lateral shifting of 1 mm (to simulate peristaltic shear) on porcine small intestine tissue (Animal Biotech Industries Inc, PA). The calibrated system noise is about 3 mN.

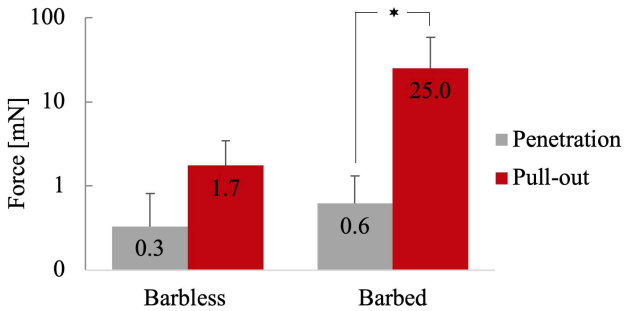

*Figure 5: Plot of penetration force (absolute) at 0.4 mm penetration depth and maximum pull-out force of barbless (n=4) and barbed microneedle (n=17, * $P < 0.05$).*

The barbed microneedles achieved a 1-2 orders of magnitudes lower penetration force with comparable pull-out force, resulting in a more than ten-fold enhancement in the PPR compared to the state-of-the-art biomimetic microneedles. These include magnetorheological drawing

honeybee stingers and reported penetration force, pull-out force, and PPR of -42 mN, 73 mN, and ~1.8 on rabbit skin, respectively [5]. Compared to those from natural honeybee stingers which reported penetration force, pull-out force, and PPR of -5.8 mN, 114 mN, and ~20 on rabbit skin, respectively [3], the barbed microneedle presents even lower penetration force, lower pull-out force, and equivalent PPR. These results suggest that the DLW-printed microneedles, benefiting from precision fabrication, demonstrate facile tissue anchoring with excellent robustness.

CONCLUSION

In this paper we have introduced bioinspired barbed microneedles fabricated via direct laser writing technology and characterized the tissue anchoring performance on porcine small intestine tissue. The high precision/fidelity structural replication of the parasitic worm proboscis plays a key role in achieving robust anchoring with exceptionally low penetration force and significantly increased pull-out force compared to penetration. The pull-out/penetration ratio demonstrated over ten-fold higher performance compared to the state-of-art barbed microneedles fabricated using other methods. The result also confirmed that microbarbs are an essential component for tissue anchoring. Overall, the ease of tissue penetration and robust anchoring strength provide great opportunities for conveniently implanting devices embed within the intestinal epithelium. The utilization of this technology will ultimately aid in the development of ingestible capsule systems, particularly for deploying resident devices [7] for extended monitoring and drug delivery in the GI tract.

ACKNOWLEDGEMENTS

This work was funded by the National Science Foundation ECCS Program (Award 1738211). The authors would like to acknowledge Terrapin Works, Maryland Nanocenter and its FabLab, as well as Bioinspired Advanced Manufacturing Laboratory at University of Maryland, College Park for their support in fabrication and characterization.

REFERENCES

[1] W. K. Cho, J.A. Ankrum, D. Guo, S.A., Chester, S.Y. Yang, A. Kashyap, G.A. Campbell, R.J. Wood, R.K. Rijal, R. Karnik, R. Langer, "Microstructured barbs on the North American porcupine quill enable easy tissue penetration and difficult removal", *Proc. Natl. Acad. Sci. U. S. A.*, vol. 109.52, pp. 21289-21294, 2012.

[2] X. Q. Kong, C. W. Wu, "Mosquito proboscis: An elegant biomicroelectromechanical system," *Phys. Rev. E.*, vol. 82.1, pp.011910, 2010.

[3] J. Ling, Z. Song, J. Wang, K. Chen, J. Li, S. Xu, L. Ren, Z. Chen, D. Jin, L. Jiang., "Effect of honeybee stinger and its microstructured barbs on insertion and pull force," *J. Mech. Behav. Biomed. Mater.*, vol. 68, pp. 173-179, 2017.

[4] O. A. Hammond, "The Proboscis Mechanism of Acanthocephalus Ranae", *J. Exp. Biol.*, vol. 45.2, pp. 203-213, 1966.

[5] Z. Chen, Y. Lin, W. Lee, L. Ren, B. Liu, L. Liang, Z. Wang, L. Jiang, "Additive Manufacturing of Honeybee-Inspired Microneedle for Easy Skin Insertion and Difficult Removal", *ACS Appl. Mater. Interfaces*, vol. 10.35, pp. 29338-29346, 2018.

[6] W. Xie, W. M. Lewis, J. Kaser, C. R. Welch, P. Li, C. A. Nelson, V. Kothari, B. S. Terry, "Design and Validation of a Biosensor Implantation Capsule Robot", *J. Biomech. Eng.*, vol. 139, pp. 081003–1, 2017.

[7] C. Steiger, A. Abramson, P. Nadeau, A. P. Chandrakasan, R. Langer, G. Traverso, "Ingestible electronics for diagnostics and therapy", *Nat. Rev. Mater.*, vol. 4.2, pp. 83-98, 2019.

[8] C. Quaglia, S. Tognarelli, E. Sinibaldi, N. Funaro, P. Dario, A. Menciassi, "Wireless robotic capsule for releasing bioadhesive patches in the gastrointestinal tract", *A. Menciassi, J. Med. Devices, Trans. ASME*, vol. 4.2, pp. 83-98, 2014.

[9] P. Griss, P. Enoksson, and G. Stemme, "Micromachined barbed spikes for mechanical chip attachment," *Sensor Actuat A-Phys.,* vol. 95, pp. 94-99, 2002.

[10] S. Tognarelli, V. Pensabene, S. Condino, P. Valdastri, A. Menciassi, A. Arezzo, P. Dario, "A pilot study on a new anchoring mechanism for surgical applications based on mucoadhesives," *Minim. Invasive Ther. Allied Technol.*, vol. 20.1, pp. 3-13, 2011.

[11] P. Li, C. Kreikemeier-Bower, W. Xie, V. Kothari, B. S. Terry, "Design of a Wireless Medical Capsule for Measuring the Contact Pressure Between a Capsule and the Small Intestine", *J. Biomech. Eng.,* vol. 139, pp. 51003, 2017.

[12] S. D. Senturia, *Microsystem design*. Springer Science & Business Media, 2007.

[13] S. Y. Yang, E. D. O'Cearbhaill, G. C. Sisk, K. M. Park, W. K. Cho, M. Villiger, B. E. Bouma, B. Pomahac, J. M. Karp, "A bio-inspired swellable microneedle adhesive for mechanical interlocking with tissue," *Nat. Commun.*, vol. 4, pp. 1702, 2013.

[14] O. M. Amin, R. A. Heckmann, N. V. Ha, "Acanthocephalans from fishes and amphibians in Vietnam, with descriptions of five new species", *Parasite*, vol. 21.53, pp. 1, 2014.

[15] E. D. Lemma*, F. Rizzi, T. Dattoma, B. Spagnolo, L. Sileo, A. Qualtieri, M. De Vittorio, F. Pisanello*, "Mechanical Properties Tunability of Three-Dimensional Polymeric Structures in Two-Photon Lithography," *IEEE Trans. Nanotechnol.*, vol. 16.1, pp. 23-31 2016.

[16] R. A. Cone, "Barrier properties of mucus," *Adv. Drug Deliv. Rev.*, vol. 61.2, pp. 75-85, 2009.

[17] M. E. V Johansson, D. Ambort, T. Pelaseyed, A. Schütte, J.K. Gustafsson, A. Ermund, D.B. Subramani, J.M. Holmén-Larsson, K.A. Thomsson, J.H. Bergström, S. van der Post, "Composition and functional role of the mucus layers in the intestine," *Cell. Mol. Life Sci.*, vol. 68.22, pp. 3635, 2011.

CONTACT

* R. Ghodssi; +1-301-405-8158; ghodssi@umd.edu