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Supplemental Information

Hydrogel Microfilaments toward Intradermal Health Monitoring

Nalin Tejavibulya, David A.M. Colburn, Francis A. Marcogliese, Kyung-Ae Yang, Vincent Guo, Shilpika Chowdhury, Milan N. Stojanovic, and Samuel K. Sia

Supplemental Figures

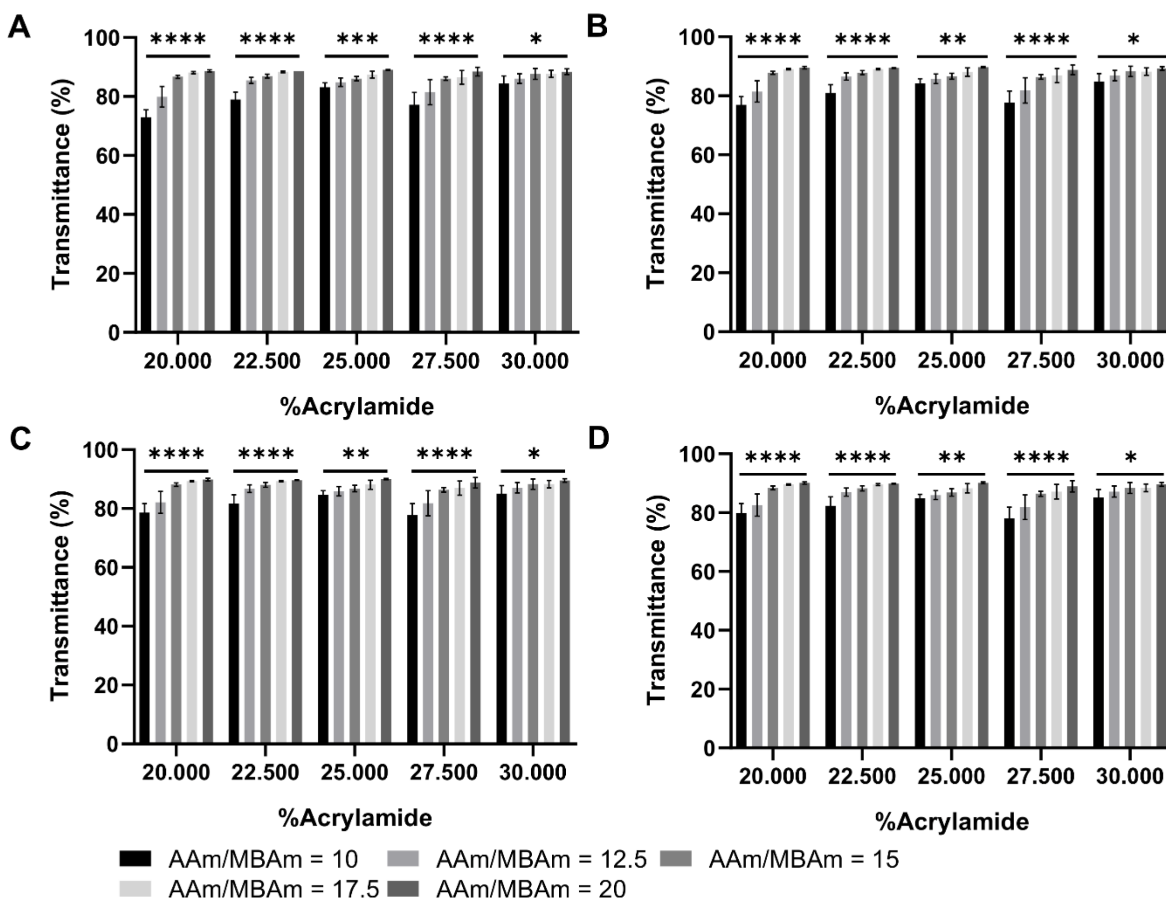

Figure S1. Percent transmittance of polyacrylamide hydrogel, related to Figure 2B. (A) Percent transmittance values of polyacrylamide disks at $\lambda = 470$ nm and (B) $\lambda = 520$ nm, the peak excitation and emission wavelengths for FAM, respectively (n=4). (C) Percent transmittance values of hydrated polyacrylamide disks at $\lambda = 550$ nm and (D) $\lambda = 580$ nm, the peak excitation and emission wavelengths for TAMRA, respectively (n=4). Data analyzed using a two-way ANOVA with Tukey post-hoc test. "ns" indicates not significant, "*" indicates significant at $p < 0.05$, "**" indicates significant at $p < 0.01$, "***" indicates significant at $p < 0.001$, and "****" indicates significant at $p < 0.0001$. Line indicates comparison between groups. Data are represented as mean ± standard deviation.

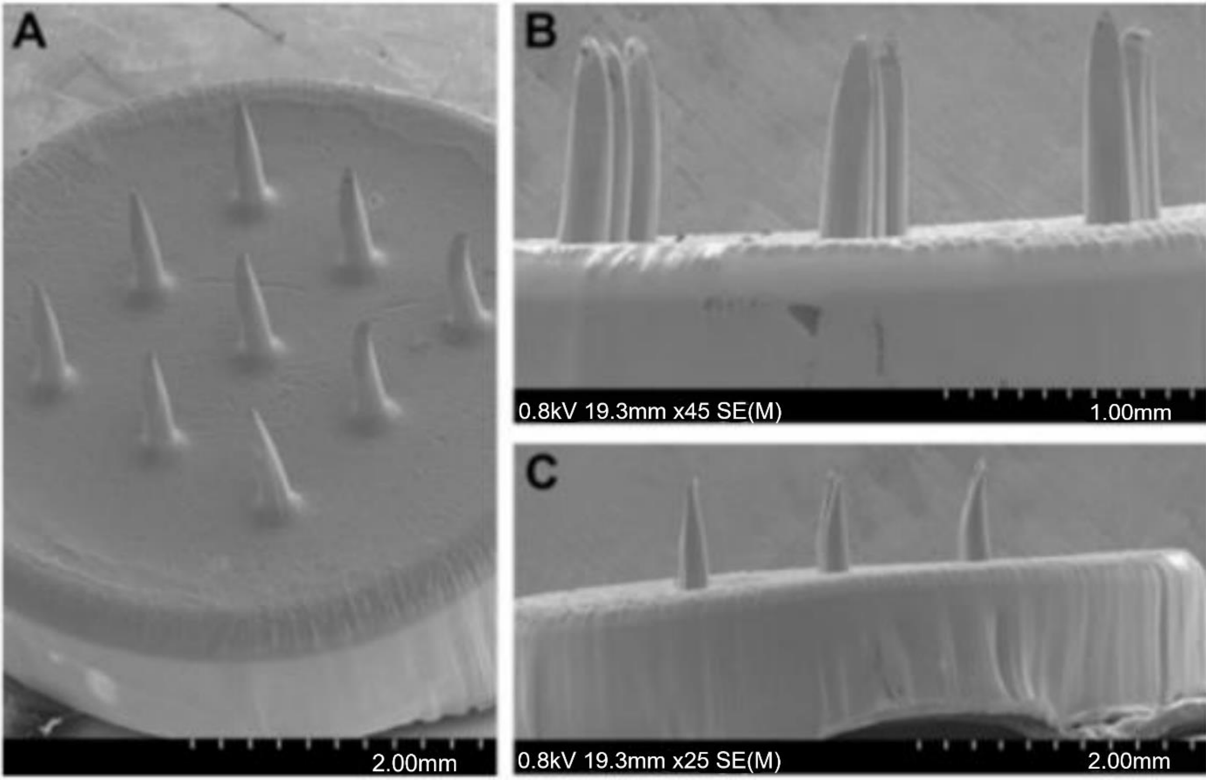

Figure S2. SEM images of microfilament array, related to Figure 2C. (A) Image at 45° relative to the base. (B) Image from the side with bevel facing forward. (C) Image from the side with bevel facing sideways.

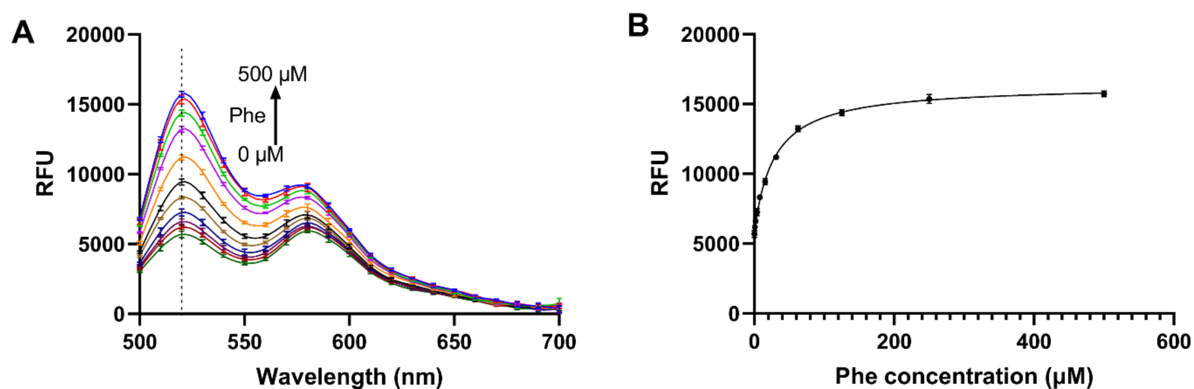

Figure S3. Concentration dependence of emission spectra and peak emission wavelength, related to Figure 3. (A) Fluorescence emission spectra of aqueous aptamer sensor exposed to different concentrations of phenylalanine solution and excited at λ = 470 nm (n=5). (B) Fluorescence intensity concentration response for aqueous aptamer sensor with excitation λ = 470 nm and emission λ = 520 nm (n=5). The curve was fit using a Padé approximant. Data are represented as mean ± standard deviation.

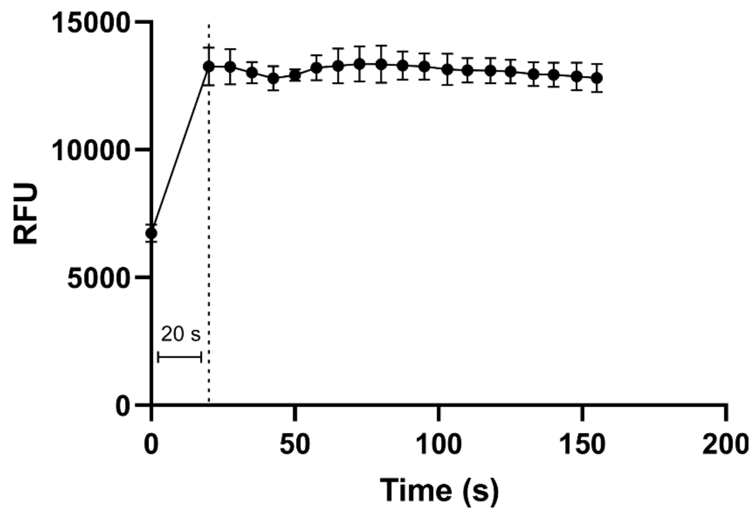

Figure S4. Response time of aqueous aptamer sensor, related to Figure 3. Fluorescence intensity for aqueous aptamer sensor with λ = 470 nm and emission λ = 520 nm after increasing phenylalanine concentration from 0 μM to 500 μM (n=3). Data are represented as mean ± standard deviation.

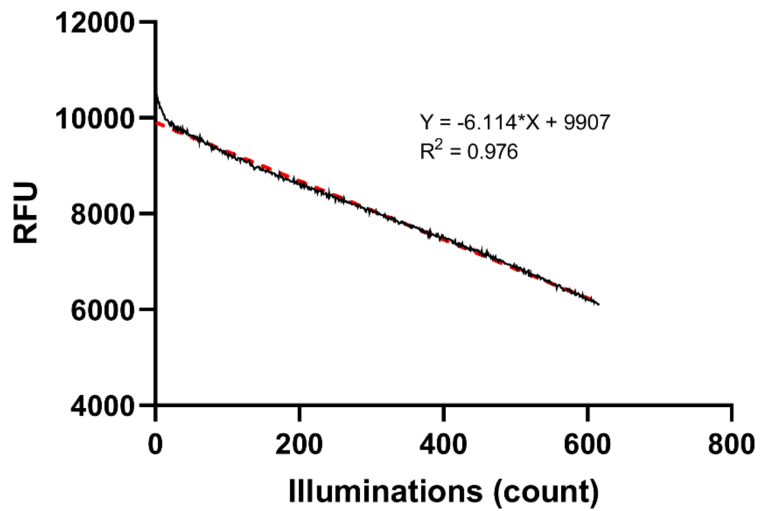

Figure S5. Photobleaching of aqueous aptamer sensor, related to Figure 3. Mean fluorescence intensity of aqueous aptamer sensor after repeated reads with excitation λ = 470 nm and emission λ = 520 nm (n=5). Red dotted line indicates the line of best fit. Data are represented as mean.

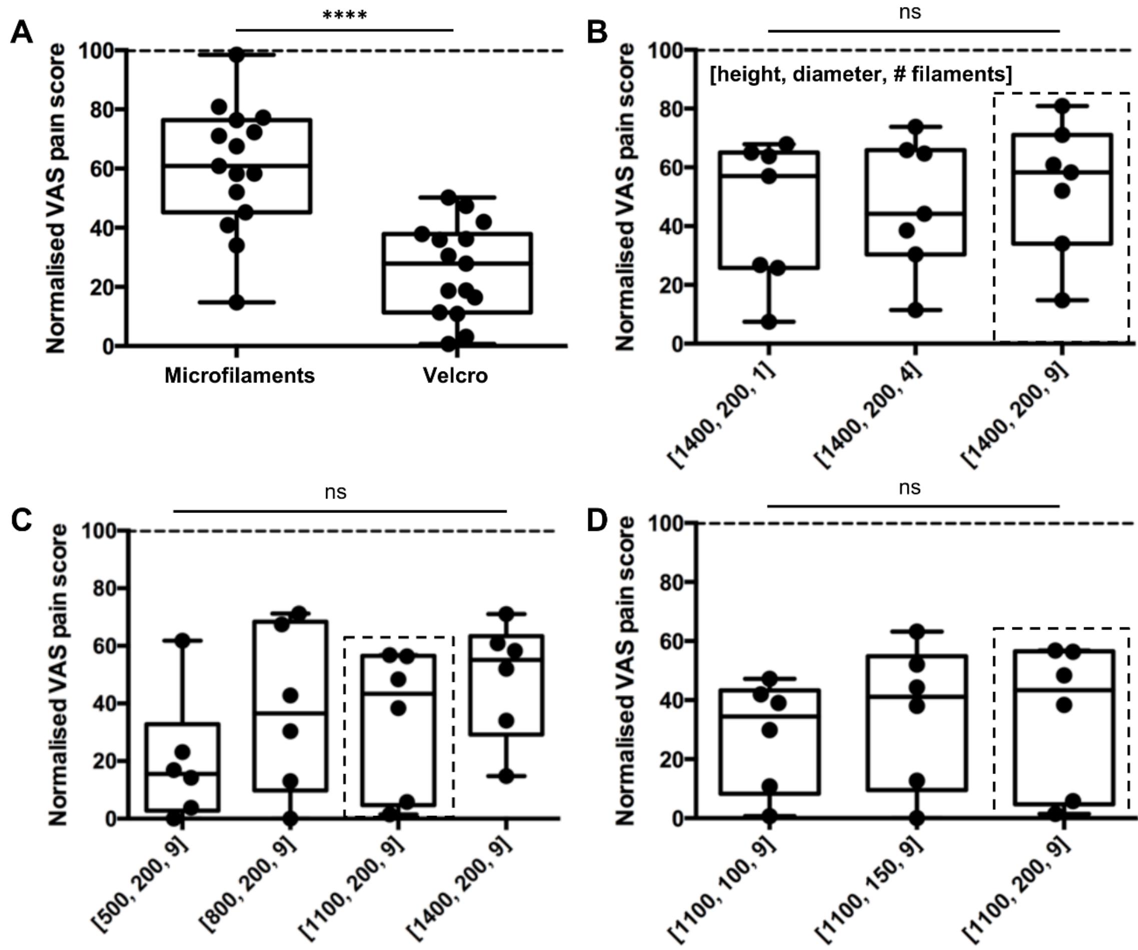

Figure S6. Normalized VAS pain score from human subjects, related to Figure 4A. (A) Box plots of VAS pain scores for 3x3 array of microfilaments or Velcro patch normalized to a 26-gauge hypodermic needle (n=15). (B) Box plots of VAS pain scores normalized to a 26-gauge hypodermic needle for microfilament arrays with variable number of microfilaments (n=7). (C) Box plots of VAS pain scores normalized to a 26-gauge hypodermic needle for 3x3 array with microfilaments of variable height (n=6). (D) Box plots of VAS pain scores normalized to a 26-gauge hypodermic needle for 3x3 array with microfilaments of variable diameter (n=6). The dotted lines indicate the design that was identified as optimal for each group. Data analyzed using a one-way ANOVA with Sidak post-hoc test. “ns” indicates not significant, and “****” indicates significant at $p < 0.0001$. Line indicates comparison between groups.

Supplemental Tables

				AAm/MBAm Ratio		
				15	17.5	20
%AAm (%w/v)	20	λ	470 nm	0.062 ± 0.003	0.055 ± 0.002	0.052 ± 0.002
			520 nm	0.057 ± 0.002	0.051 ± 0.001	0.048 ± 0.002
			550 nm	0.055 ± 0.003	0.049 ± 0.001	0.047 ± 0.002
			580 nm	0.054 ± 0.003	0.048 ± 0.001	0.045 ± 0.002
	25	λ	470 nm	0.065 ± 0.004	0.059 ± 0.006	0.051 ± 0.001
			520 nm	0.063 ± 0.005	0.056 ± 0.007	0.047 ± 0.001
			550 nm	0.062 ± 0.005	0.055 ± 0.008	0.046 ± 0.001
			580 nm	0.061 ± 0.006	0.054 ± 0.008	0.045 ± 0.001
	30	λ	470 nm	0.058 ± 0.009	0.057 ± 0.006	0.054 ± 0.005
			520 nm	0.054 ± 0.009	0.055 ± 0.006	0.050 ± 0.003
			550 nm	0.054 ± 0.009	0.054 ± 0.006	0.048 ± 0.003
			580 nm	0.054 ± 0.009	0.054 ± 0.006	0.048 ± 0.003

Table S1. Absorbance of polyacrylamide at 470 nm, 520 nm, 550 nm, and 580 nm, related to Figure 2B. Absorbance for polyacrylamide formulations with variable acrylamide percentage (%AAm) and ratio of acrylamide to N’N-methylenebisacrylamide crosslinker (AAm/MBAm). Data are represented as mean ± standard deviation.

Transparent Methods

Procedure for Measuring Hydrogel Absorbance

Polyacrylamide precursor solutions consisting of acrylamide (AAm) (Polysciences; Warrington, PA), 2% w/v cross-linking agent N'N-methylenebisacrylamide (MBAm) (Sigma-Aldrich, Allentown, PA), and photoinitiator 2-hydroxy-2-methylpropiophenone (Darocur 1173; BASF Corporation; Florham Park, NJ) were prepared in deionized water. Polyacrylamide disks (diameter: ~6.4 mm, thickness: ~6 mm) of different compositions (%AAm of [20, 22.5, 25, 27.5, 30] and AAm/MBAm ratios of [10, 12.5, 15, 17.5, 20]) were cast in a 96-well flat bottom plate and photopolymerized under a collimated UV light source (365 nm, power ~1.8 mW/cm^2) (Omnicure series 2000; Lumen Dynamics Group; Mississauga, ON) for 10 seconds. The absorbance was then read at the peak excitation and emission wavelengths for FAM, 470 nm and 520 nm, and TAMRA, 550 nm and 580 nm, using a plate reader (Synergy H1; BioTek; Winooski, VT). The reported absorbance values of the hydrogels were computed by subtraction of the average absorbance due to the well plate surface. Percentage of transmittance values can also be inferred from the absorbance values by the following equation:

$$\text{Absorbance} = 2 - \log_{10} \%\ \text{Transmittance}$$

A two-way ANOVA was used to assess the significance of the %AAm and AAm/MBAm ratio. Multiple t-tests with the Tukey correction was used to compare groups.

Procedure for Determining Young's Modulus and Shrinkage Percentage

Polyacrylamide disks of different compositions (%AAm of [20, 25, 30] and AAm/MBAm ratios of [15, 17.5, 20]), cast in a 96-well plate, were desiccated and stored at room temperature for at least 2 days following polymerization to allow for complete drying prior to testing. Before performing mechanical testing, the dimensions of each disk were measured using a Vernier caliper. These measurements were used to calculate the shrinkage percentage. To measure the stiffness of desiccated polyacrylamide, compression test was performed using a materials testing machine (Instron 8841 Microtester; Instron; Norwood, MA), equipped with stainless steel platens and a 250-lb load cell. Using integrated Instron software, the platens were brought into contact with the disk sample on the stage until a 10 gf (0.098 N) preload was reached. Then compression over a displacement of 500 μm was applied at a rate of 0.001 mm/s. The integrated software was used to track the displacement of the platens and the readings of the load cell. This procedure was repeated to determine the Young's modulus for PEEK disks using a 450-lb load cell.

To determine the Young's modulus of the hydrated hydrogel, a custom unconfined compression device was used equipped with a 250-gf load cell. After reaching a preload of 2 gf, the hydrogel was compressed to 90% of the unloaded thickness at a strain rate of 0.05%.

For all samples, the Young's modulus was calculated by dividing the difference between the preload and equilibrium load by the sample area times the percent strain.

$$\text{Young's Modulus} = \frac{\text{Equilibrium load} - \text{Preload}}{\text{Area}} \cdot \frac{1}{\%\ \text{Strain}}$$

A two-way ANOVA was used to assess the significance of the %AAm and AAm/MBAm ratio with a Tukey post-hoc test applied for multiple comparisons.

Procedure for Determining Diffusion Coefficient for 2-NBDG

Glass slides were treated with 0.48% v/v 3-(trimethoxysilyl)propyl methacrylate (TMSM) (Sigma-Aldrich; Allentown, PA) and 2.90% glacial acetic acid in ethanol for 3 minutes and then rinsed with ethanol. Polyacrylamide hydrogel with 30% AAm and AAm/MBAm ratio of 15 was polymerized to form 5 x 5 x 0.5 mm slabs on the glass slide. The hydrogel was incubated overnight in a 8 x 8 x 5 mm well containing a solution of fluorescent glucose analog 2-(N-(7-nitrobenz-2-oxa-1,3,-diazol-4-yl)amino)-2-deoxyglucose) (2-NBDG) (Thermo Fisher Scientific; Eugene, OR). Fluorescence recovery after photobleaching (FRAP) was then performed by using a confocal microscope (Leica TCS SP5 Confocal and Multi-Photon Microscope; Wetzlar, Germany). Firstly, a circular region of diameter 20 μm was bleached through the

fluorescent hydrogel using 488 nm Argon laser set at 95% power. The hydrogel was subsequently images at a lower power of 6% as to avoid further bleaching. Fluorescence recovery in the bleached region was imaged every 0.229 seconds for 45 seconds, allowing for determination of the lateral transport rate, in the idealized case of pure two-dimensional diffusion monitored by a uniform circular disc profile. The diffusion coefficient is determined from an experimental recovery curve $\hat{f}$, using the following equation:

$$D = \left(\frac{w^2}{4\tau_{1/2}}\right)\gamma_D$$

where w is the radius of the disc, γ_D is 0.88, and $\tau_{1/2}$is the time for which $\hat{f}(\tau_{1/2}) = \frac{1}{2}$.

Four separate FRAP experiments were performed for four independently prepared samples. The mean diffusion coefficient from the repeated FRAP experiments was taken to represent the sample, and the mean and standard deviation of the independently prepared samples were computed.

Fabrication of PEEK Microfilament Arrays for Human Subject Assessment
Microfilament arrays were designed using SolidWorks CAD Software (SolidWorks, Concord, MA) and fabricated via CNC milling of polyether ether ketone (PEEK) (McMaster-Carr; Robbinsville, NJ), a medical-grade biocompatible plastic with high mechanical strength, using Haas MiniMill 4x (Haas Automation; Oxnard, CA). Each array has a diameter of 7 mm and contains 1, 4, or 9 microfilaments in a 1x1, 2x2, or 3x3 configuration; the microfilaments are 500, 800, 1100, or 1400 µm tall, 100, 150, or 200 µm in diameter, have a 74° diamond-shaped bevel, and are spaced 1500 µm apart tip-to-tip. To sterilize, each microfilament array was disinfected by sonication in two fresh baths of 70% ethanol, air-dried, and steam sterilized at 121°C for 30 minutes. After sterilization, the PEEK microfilament devices were left in the sealed pouch in a sterile environment until use.

Pain Assessment and Healing Response in Human Subjects
All pain assessments on human subjects were approved by the Institutional Review Boards at Columbia University Medical Center, and research was carried out with informed consent from the subjects. Human subjects were recruited form the students and staff population at Columbia University. Susceptibility to hypodermic scarring was used as the subject exclusion criterion. Overall, 6 males and 9 females (ages 19-56) participated in this study. The level of pain experienced due to the application or insertion of the device in question was measured using the visual analogue scale (VAS), as previously described (Hawker et al., 2011). Controls for this experiment include: 26-guage hypodermic needle as the positive control, a flat circular surface of PEEK rod of 7 mm diameter as the negative control; and the hook surface of a hook-and-loop fastener as a comparison. Application or insertion of the device was performed manually on randomized locations of the subject's volar forearms, previously cleaned with isopropanol swabs by the investigator. All experiments began with the insertion of the PEEK microfilament array, the negative, and the positive controls to help the subjects calibrate their response to the range of sensations to be encountered on the VAS scale. Each treatment lasted ~10 seconds, and the pain level indicated on the VAS scale by the subject for each treatment was recorded. All insertions were performed in triplicate for every subject, and in random sequence. For each subject, average raw VAS pain score for each of the devices assessed were calculated and normalized to the average raw hypodermic 26-guage needle pain score to account for the variability in a subject's perception and to provide a common reference point.

6 of the 15 subjects were used to determine the effects of microfilament length, diameter, and the number of microfilaments in an array on insertion pain. The study was carried out in two stages, in order to avoid carryover effects from too many insertions. In the first stage, the effects of microfilament length and number of microfilaments in an array were investigated. The PEEK microfilament devices tested in this stage included (denoted [length (µm), diameter (µm), number of microfilaments]) [500, 200, 9]; [800, 200, 9]; [1400, 200, 9]; [1400, 200, 4]; and [1400, 200, 1]. In the second stage, the effects of diameter

were investigated using the devices of [1100, 100, 9]; [1100, 150, 9]; and [1100, 200, 9]. The two stages were separated by a minimum of 20 minutes of break.

To assess the healing response after insertion and removal of PEEK microfilament arrays, images of the skin surface were capture at multiple points after device removal.

A one-way ANOVA was used to assess the significance of the designs and geometries with a Sidak post-hoc test applied for multiple comparisons.

Fabrication of Polyacrylamide Microfilaments Arrays

Positive microfilament array molds were designed using SolidWorks CAD Software (SolidWorks; Concord, MA) and fabricated via CNC milling machinable acrylic plastic (McMaster-Carr; Robbinsville, NJ) using Haas MiniMill 4x. Silicone negative mold is then replicated from the positive mold with Dragon Skin platinum silicone (10 Medium; Smooth-On; Macungie, PA) with the two components mixed in the ratio of 1:1, degassed and cured on the acrylic molds at 60°C for 4 hours. The cured silicone mold is removed from the acrylic master, rinsed in water and 70% ethanol, and wet autoclaved. Microfilament arrays were prepared by casting polyacrylamide precursor solution in the negative silicone mold and photopolymerized under a collimated UV light source.

Measurement of Microfilament Penetration and Failure Forces

Full-thickness human cadaver skin was obtained from the Columbia University Anatomical Gift Program with approval from the Columbia University Medical Center Institutional Review Boards, and used according to the Declaration of Helsinki Principles. The skin was derived from the abdominal region. Full ethical and regulatory approval was obtained from Columbia University Medical Centre Institutional Review Boards for use of all these tissues and the subsequent experimentation. The skin samples were stored at -20°C until use. Before use, the skin tissue was allowed to thaw at room temperature for 2 hours, and cut into ~2 x 2 cm pieces followed by immersion in PBS for 2 hours. A flat section of skin tissue was affixed using cyanoacrylate glue to a test fixture and mounted on the base platen of the Instron 8841 Microtester. The microfilament array (3x3 array and 7 mm diameter) or a flat polymer disk was glued onto the opposing test fixture and fixed on the upper platen of the mechanical tester. Test samples were brought into contact with the tissue until a 10 gf (0.098 N) preload was reached. The samples were then displaced at a rate of 0.02 mm/s, and the force was recorded for insertion. The samples were displaced at the same rate of 0.02 mm/s in the opposite, and the force was recorded for removal.

Mechanical failure of microfilaments was considered due to axial loading and traverse loading. To measure the force a microfilament can withstand before failure under an axial load (i.e. force applied parallel to the microfilament axis), the microfilament array on the upper platen was pressed against the rigid metal surface of the base platen at 1 mm/s. Upon failure, the force suddenly dropped; the maximum force applied immediately before dropping was interpreted as the force of needle failure. Microfilaments were examined by microscopy (SZX2-ILLK, Olympus) before and after failure testing to determine the mode of failure, e.g. buckling failure due to inelastic or elastic instability. Data were discarded if only some of the microfilaments were broken. Data are reported as the force per microfilament for failure.

Interstitial Fluid Sampling in Hairless Rats

Polyacrylamide microfilament arrays were fabricated using a polymer composition of 30% w/v AAm, 2% w/v MBAm, 0.5% v/v Darocur 1173. The microfilament arrays were placed on a CD hairless rat (Charles River; Raleigh, NC) and allowed to equilibrate. The rat was anaesthetized by isoflurane inhalation (Isothesia; Henry Schein Animal Health; Dublin, OH) delivered at 1.5 mL/h via an E-Z Anesthesia (E-Z Systems Corp; Palmer, PA), and its body temperature was maintained at 37°C using a warm water recirculating heat pad. The microfilament arrays were inserted into the skin by a gentle press and secured in position with skin adhesive (Tegaderm; 3M; Appleton, WI). After being worn on rat skin for 1 hour, the microfilament arrays were removed and subsequently treated with silver nitrate solution (Sigma-Aldrich; Allentown, PA) to detect the presence of chloride ions from the interstitial fluid. For visualization purposes, the red and yellow channels were boosted to highlight the silver deposits. All animal studies were approved by the Columbia University Institutional Animal Care and Use Committee.

Histological Preparation
Polyacrylamide microfilament arrays were fabricated using a polymer composition of 30% w/v AAm, 2% w/v MBAm, 0.5% v/v Darocur 1173. The microfilament arrays were placed on a CD hairless rat. All microfilament arrays were worn on rats for at least 24 hours before removal. After the rats have been sacrificed, the insertion sites were excised from the bulk skin with a scalpel. Samples were obtained immediately, 30 mins, 1 hour, 2 hours and 4 hours after removal of microfilament array from hairless rat skin. The isolated skin pieces were placed in cryostat molds embedded with optimum cutting temperature (OCT) media (Tissue-Tek, Torrance, CA). The skin was fixed in OCT by freezing the sample on dry ice. Frozen skin samples were sliced into 12-μm thick sections (Cryo-star HM 560MV, Microm, Waldorf, Germany). The skin sections were stained with hematoxylin and eosin using an automated staining machine (Leica Autostainer XL, Nussloch, Germany). After staining, the sections were covered with glass slides sealed with cytoseal 60 (low viscosity, Richard-Allan Scientific, Kalamazoo, MI). The sections were dried overnight before taking images under the microscope (Leica TCS SP5 Confocal, Wetzlar, Germany). All animal studies were approved by the Columbia University Institutional Animal Care and Use Committee.

Aqueous Emission Spectra and Peak Emission Curve
Oligonucleotide aptamer with L-phenylalanine binding site, 6-carboxyfluorescein (FAM) and 6-carboxytetramethylrhodamine (TAMRA) fluorophore reporting unit, and 5'-acrydite modification (Integrated DNA Technologies; Coralville, IA) was prepared to 200 nm using phosphate buffered saline with 2 mM $MgCl_2$ (PBS). The aptamer solution was placed in boiling water for 5 minutes then allowed to cool for 30 minutes. Aliquots of the prepared aptamer sensor were added to equal volume aliquots of phenylalanine (Sigma-Aldrich, Allentown, PA) solutions prepared via serial dilution from 1000 μM to 1.95 μM. The aptamer-phenylalanine solutions were allowed to react for 40 minutes before being pipetted into a black 96-well clear, flat bottom plate. The fluorescence was read in a plate reader with λ_{ex} = 470 nm and λ_{em} scanned between 500 and 650 nm. The fluorescence intensity at 520 nm was used to construct the curve showing concentration dependence at peak fluorescence emission.

Aqueous Response Time
200 nM aptamer sensor solution was prepared in PBS. The aptamer solution was placed in boiling water for 5 minutes then allowed to cool for 30 minutes. Aliquots of aptamer solution were combined with equal volume aliquots of PBS and pipetted into a 96-well plate. The fluorescence was read in a plate reader with λ_{ex} = 470 nm and λ_{em} = 520 nm. These readings served as the values for t = 0 seconds. Next, aliquots of the 200 nM aptamer solution were pipetted into the 96-well plate. Equal volume aliquots of 500 μM phenylalanine solution was then added into the same wells. The plate was quickly placed in the plate reader, and the fluorescence was read with λ_{ex} = 470 nm and λ_{em} = 520 nm with a read taken every 7.5 sec. A stopwatch was used to determine the time between addition of the phenylalanine aliquot and the first fluorescence reading.

Aqueous Photobleaching
200 nM aptamer sensor solution was prepared in PBS. The aptamer solution was placed in boiling water for 5 minutes then allowed to cool for 30 minutes. Aliquots of aptamer solution were combined with equal volume aliquots of PBS with 1000 μM phenylalanine and allowed to react for 40 minutes. The solution was then pipetted into a 96-well plate. The fluorescence was read in a plate reader with λ_{ex} = 470 nm and λ_{em} = 520 nm. Fluorescence readings were taken repeatedly to assess the effects of photobleaching.

Hydrogel Emission Spectra and Peak Emission Curve
A polyacrylamide precursor solution of 30% w/v AAm, 2% w/v MBAm, and 100 nM aptamer sensor was prepared in PBS. The precursor solution was placed in boiling water for 5 minutes then allowed to cool for 30 minutes. Next, 0.5% v/v of Darocur 1173 was added. The monomer solution was polymerized into disks ~5.6 mm diameter and ~2.3 mm height. The disks were then placed in PBS with the specified phenylalanine concentration prepared via serial dilution from 500 μM to 0.98 μM and soaked overnight. The disks were then removed from the phenylalanine solution, dried with a Kimwipe, and placed in a 96-well plate. The fluorescence was read in a plate reader with λ_{ex} = 470 nm and λ_{em} scanned between 500 and 650 nm. The fluorescence intensity at 520 nm was used to construct the curve showing concentration dependence at peak fluorescence emission.

Hydrogel Response Time
A polyacrylamide precursor solution of 30% w/v AAm, 2% w/v MBAm, and 100 nM aptamer sensor was prepared in PBS. The precursor solution was placed in boiling water for 5 minutes then allowed to cool for 30 minutes. Next, 0.5% v/v of Darocur 1173 was added. The monomer solution was polymerized into disks of ~6.4 mm diameter and ~1 mm height in a 96-well flat bottom plate. PBS was added to a subset of disks, and the fluorescence was read in a plate reader with λ_{ex} = 470 nm and λ_{em} = 520 nm. These readings served as the values for t = 0 seconds. Next, 500 μM phenylalanine solutions were added to the wells with the remaining disks. The fluorescence was read with λ_{ex} = 470 nm and λ_{em} = 520 nm with a read taken every 70 seconds.

Hydrogel Leaching
A polyacrylamide precursor solution of 30% w/v AAm, 2% w/v MBAm, and 100 nM aptamer sensor was prepared in PBS. The precursor solution was placed in boiling water for 5 minutes then allowed to cool for 30 minutes. Next, 0.5% v/v of Darocur 1173 was added. The monomer solution was polymerized into disks of ~5.6 mm diameter and ~2.3 mm height. A subset of disks was placed in a small volume of PBS (~50 μL per disk) and allowed to soak for 2 hours to ensure they were fully hydrated. Next, the disks were removed from the PBS, dried with a Kimwipe, and placed in a 96-well plate. The fluorescence was read in a plate reader with λ_{ex} = 470 nm and λ_{em} = 520 nm. The remaining disks were placed in an excess of PBS and soaked overnight. The next day, a subset of disks were again removed and the fluorescence was read. The remaining disks were then placed in a fresh excess of PBS. This procedure was repeated 3 more times with the disks read and the PBS changed daily. This experiment was repeated as specified, but the aptamer sensor was substituted with a sensor without the 5'-acrydite modification to serve as a control. A two-tailed t-test was used to compare the endpoint fluorescence between the two groups.

Hydrogel Reversibility
A polyacrylamide precursor solution of 30% w/v AAm, 2% w/v MBAm, and 100 nM aptamer sensor was prepared in PBS. The precursor solution was placed in boiling water for 5 minutes then allowed to cool for 30 minutes. Next, 0.5% v/v of Darocur 1173 was added. The monomer solution was polymerized into disks of ~5.6 mm diameter and ~2.3 mm height. The disks were then placed in PBS and allowed to soak for 2 hours to ensure they were fully hydrated. Next, a subset of disks was removed from the PBS, dried with a Kimwipe, and placed in a 96-well plate. The fluorescence was read in a plate reader with λ_{ex} = 470 nm and λ_{em} = 520 nm. The remaining disks were then transferred to PBS with 250 μM of phenylalanine and allowed to soak for 1 hour. The disks were then removed and placed in a fresh solution of PBS with 250 μM of phenylalanine and soaked for an additional hour. A subset of disks was then removed, dried with a Kimwipe, and placed in a 96-well plate. The fluorescence was read in a plate reader with λ_{ex} = 470 nm and λ_{em} = 520 nm. This procedure was repeated for the remaining reads at different phenylalanine concentrations in the order of 500 μM, 250 μM, 0 μM, 250 μM, 500 μM.

Statistical Analysis
Statistical tests were calculated in GraphPad Prism 8.1. The details of the statistical tests are indicated in the respective figure legends. Where data was assumed to be normally distributed, values were compared using a one-way ANOVA for single variable with a Sidak post-hoc test applied for multiple comparisons. Where data was assumed to be normally distributed, values were compared using a two-way ANOVA for more than one variable with a Tukey post-hoc test applied for multiple comparisons.