Electronic Supporting Information for

An implanted pH sensor read using radiography

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Detailed Experimental Method

Materials: Chemicals

Acrylic acid (AAc) 99%, anhydrous, Poly(ethylene glycol) diacrylate – average M_n 700 (PEGDA700) containing 100 ppm MEHQ and 300 ppm BHT as inhibitor, N, N- dimethyl formamide (DMF), and phosphate buffer saline (PBS) were obtained from Sigma-Aldrich Co. (Saint Louis, MO); n-Octyl acrylate (n-OA) containing 400 ppm MEHQ as inhibitor was purchased from Scientific Polymer Products, Inc. (Ontario, NY); and N, N'- methylene bis acrylamide (MBAAm), and 2-oxoglutaric acid (OGAc) was received from Wako Pure Chemical Industries Ltd (Richmond, VA). All the chemical components used in making the pre-gel solution were used as received. To prepare the synthesis reaction bath for the sensor film, flint glass plates, silicone rubber or polytetrafluoroethylene (PTFE) film, and binder clips were used. Reference standard pH buffers (BDH) ranging from 2 to 11 were purchased from VWR Analytical (Bridgeport, NJ). Tryptic soy broth (TSB) was obtained from EMD Millipore, (Billerica, MA, USA), and Staphylococcus aureus cultures (Seattle strain) were generously gifted by Dr. Mark Shirtliff (University of Maryland). DePuy Synthes® LCP 4.5 mm proximal tibial plates (DePuy Synthes Biomaterials, West Chester, PA) were used for fixation of a simulated bone model and a human cadaver tibia. Human cadaver tibia specimens were acquired through the Hawkins Foundation (Greenville, SC). Orthopedic plates were attached to the cadaver by an orthopedic surgeon, with the procedures and radiology performed at the surgical training center in the Patewood Memorial Campus of Greenville Health System Medical University Center. Composite bone mimics (Sawbones® 3rd generation) were acquired from Pacific Research Laboratories, Inc. (Vashon Island, WA).

pH sensitive hydrogel synthesis

To synthesize the required hydrogel film a solution was prepared by mixing the monomers, AAc (10 wt%) and n-OA (5 wt%), the chemical cross-linker, PEGDA700 (1 wt%), and the UV initiator, OGAc (0.1 wt%), together in DMF solvent. For some hydrogels, a few drops of red dve were added to the hydrating solution after synthesis for visualization purposes. Next, the precursor solution was degassed by bubbling with N₂ for 5 minutes and poured into the reaction cell, where a rectangular Silicone rubber frame of 500 µm and 350 µm thickness was sandwiched between a pair of parallel flint glass plates. The glass plates were scrubbed with Alconox[®] detergent (Sigma-Aldrich Co., St. Louis, MO), rinsed with deionized (DI) water, dried, and soaked in a glass cleaner solution of 50 g/L NaOH in 50% EtOH before each use. The rubber spacer was also scrubbed with Alconox[®] and rinsed with DI water beforehand. Next, under an inert nitrogen atmosphere maintained in a Cleatech[®] 2100-4-C glove box (Cleatech, LLC, Santa Ana, CA) with attached oxygen analyzer and a Cleatech A21-HM-OA Nitrogen Purge controller, a photo polymerization reaction was performed using UV irradiation (365 nm) from both sides of the reaction cell at a temperature of approximately 45°C. After completion of the prolonged (6 hour) polymerization, hydrogel films of approximately 40 mm \times 40 mm \times 0.5 mm in size were obtained. The hydrogel films were subsequently immersed in 70% ethanol to remove the DMF and for spontaneous hydration. The ethanol and water mixture was changed once daily for at least 5 days for satisfactory removal of the un-reacted monomers and initiators in the hydrogel film. The swollen hydrogels were finally transferred and incubated in PBS 1x solution of pH 7.4. To obtain test responsive material, the hydrogel film swollen in PBS solution was cut into a disk dimensions using a metallic hole punch. A small tungsten rod, diameter 0.25 mm, was cut to 4 mm length and incorporated into the poly (AAc-co-n-OA) hydrogel disk to track the deformation (i.e., expansion and contraction) of the polymer network due to changes in the pH of the environment.

Hydrogel sensor size vs. pH calibration curve

A hydrogel disk was fully immersed in a series of pH buffers ranging from 2 to 11 at room temperature (25 °C) and its diameter measured to generate a calibration curve (main text Figure 3a). Initially it measured approximately 13 mm diameter, 1 mm thick when stored in PBS 1x (pH 7.4). To exchange buffer, the sensor was first removed from its old buffer, rinsed three times with the new buffer and then left in 25 mL of the same new buffer for > 6 hours for equilibrium to be established and the disk's diameter to stabilize. Next, photographs of the hydrogel were acquired with the Q-scope[®] camera, and the diameter was determined using the ImageJ software package. The hydrogel diameter was also measured at 40 °C and compared to the room-temperature data (Figure S2).

In vitro swelling experiments

a) Effects of ionic-strength

The sensor was tested in pH buffers where the solvent ionic concentrations were varied in the range of physiological conditions. In this experiment, the concentration of NaCl was varied from 120 mM to 160 mM to cover the lower and upper limits of the expected physiological range (Figure S3). Other analytes such as K^+ and PO_4^{3-} were not varied but maintained at physiologic concentrations of 4.1 mM and 2.1 mM respectively.

b) Hydrogel swelling and deswelling kinetics

A hydrogel disk was first incubated in pH 4.0 buffer. It was then rinsed and immersed in pH 7.4 buffer to onset swelling. Time lapse images were taken at 5 min intervals for approximately 1.5 hours with a microscopic Q-scope[®] camera (Euromex Microscopen BV, Arnhem, the Netherlands), and the chemical-sensing hydrogel diameter with respect to time *d*(t) was measured by analyzing the recorded images using the ImageJ software package (National Institutes of Health, Bethesda, MD). The measured diameter of the sensor was plotted against time, thus producing the swelling kinetics curve (Figure S4a). Similarly, to obtain the deswelling curve (Figure S4b), the sensor swollen in pH 7.4 was transferred into the pH 4.0 buffer. Swelling and deswelling experiments were repeated successively to check the reversibility of the hydrogel response (main text Figure 3b). In a subsequent experiment, both swelling and deswelling measurements were carried out after mounting the sensor to an orthopedic implant fixed to a prototype leg bone; the displacement of an indicator was measured with respect to a scale consisting of reference holes of consistent size and fixed center-to-center spacing equal to the hole diameter (with every other hole laterally displaced).

Response in a growing bacteria culture suspension

The hydrogel sensor was removed from the PBS storage buffer of pH 7.4 and sterilized by rinsing with 70 % ethanol followed by exposure to UV light (254 nm, 125 μ W/cm²) in a biosafety cabinet for 60 minutes. The hydrogels were wet at the completion of UV exposure and were incubated again in sterile PBS (pH 7.4). Next, *S. aureus* was cultured in sterile TSB supplemented with 1% glucose. A hydrogel disk was then removed from the PBS solution and

gently transferred to a culture plate containing sterile TSB using sterile forceps. Next, 100 μ L of *S. aureus* (approximately 10³ cells) was added to the TSB (2.5 mL and pH 7.2), and the culture was made uniform by shaking softly. Finally, the bacterial culture containing the hydrogel sensor was incubated at 37 °C. Every hour thereafter, 10 μ L of the bacterial culture was taken from the plate, its pH was measured, and an image of the hydrogel was captured to determine the overall size (*d*_t) change. A pocket pH electrode (model:H135 minilab, HACH, Loveland, CO) was used to measure pH of the cultures and control media. This experiment was continued until the pH stabilized. A similar hydrogel disk maintained in a well containing only TSB without bacteria acted as a negative control. Figures 4 and S5 of this experiment are provided in the main text and herein ESI, respectively. A similar culture experiment was conducted again with multiple hydrogels (three gel disks for cultures and three for controls) to check the reproducibility of pH responsive behavior of the sensor. A micro pH electrode (model: 9863BN, Thermo Scientific, Chelmsford, MA) was used with performing calibration to measure pH, changing in cultures and constant in controls.

X-ray imaging of sensor on a plated cadaveric tibia

The hydrogel disk swollen in PBS (pH 7.4) was placed in the custom designed metal well mounted to an orthopedic plate fixed by a surgeon on a human cadaveric tibia. A radiopaque tungsten rod approximately 4.0 mm long and 0.25 mm in diameter was adhered to the edge of the gel sensor such that the rod could translate concurrently when the hydrogel contracted or expanded due to deswelling or swelling, respectively (main text Figure 5). The metal well contained a radiopaque scale prepared by making through-holes in two parallel columns so that the displacement of the tungsten rod on the sensor boundary could be visualized and quantified. Each hole in the radiopaque scale had a diameter of 500 µm and spacing between the centers of adjacent holes was 500 µm. The hydrogel disk was secured to the well using a pin press-fitted to the base of the well. Sensor response at given pH buffers was recorded radiographically using a Ziehm Vision R mobile fluoroscopic C-arm (Ziehm Imaging Inc., Orlando, FL). The experiment using the Sawbones[®] in the Godley Snell Animal Center (Figure S9) used a Tingle 325 M X-ray unit system (TXR, Tingle X-Ray, LLC, East Cottondale, AL) for imaging and fine resolution litmus paper for measuring the solution pH. Cadaver specimens were obtained from the Hawkins Foundation (Greenville, SC) through Restore Life USA (Elizabethton, TN), a nonprofit donation program, and donors had consented their body to be used for medical education and research in accordance with the Uniform Anatomical Gift Act (UAGA).



Comparison between poly(AAc-co-n-OA) and polyAAc hydrogel disk swelling ratios measured in standard reference pH buffers. Lines show regression using a Henderson-Hasselbalch model. Inclusion of PEGDA700 and n-OA in the hydrogel increased the maximum swelling ratio and shifted the calibration curve transition range to higher pH.



Comparison between poly(AAc-co-n-OA) hydrogel disk response in TSB bacterial culture media at 40 °C and response in standard pH buffer solutions at 25 °C. NaCl concentration in TSB was maintained at approximately 100 mM.



Effect of ionic strength on hydrogel response: Hydrogel disk diameter in TSB with various pH and NaCl concentrations compared with the standard buffer calibration plot.



Time dependence of poly(AAc-co-n-OA) hydrogel disk diameter after changing buffer pH. (A) Swelling (expansion) after switching from pH 4.0 buffer to 7.4. (B) deswelling (contraction) after switching from pH 7.4 to 4.0. The expanding/contracting hydrogel diameter (d_t) is shown as a function of time and fit with an exponential. The hydrogel was initially approximately 13 mm in diameter and 1 mm thick in pH 7.4 buffer solution.



Photographs of 1 mm thick pH-sensitive hydrogel disks in *S. aureus* culture recorded in time as the pH decreased. Initial hydrogel diameter in TSB (pH 7.2) was approximately 13 mm. Diameter was estimated from images, using a mm scale ruler in the image to calibrate measurement.



Changes in mean hydrogel diameter for a set of 5 hydrogels incubated in PBS buffer at 25 °C for 13 days. Initial diameter was 13.86 mm \pm 0.018 (day 1) and diameter at day 13 was 13.88 mm \pm 0.016, an insignificant length change (0.14%, corresponding to 0.01 pH unit).



Poly(AAc-co-n-OA) hydrogel disk response after 10 days incubation period at 37 °C in either i) sterile TSB media, ii) harsh reactive oxygen species environment (TSB media containing 1 mM CuSO₄ and 50 mM H_2O_2), or iii) *S. aureus* culture in TSB media. Regression line is the calibration curve calculated from hydrogel response in standard pH buffer solutions at 25 °C. Vertical axis data in this plot was normalized by the hydrogel diameter in pH 7.4 for all the cases.



Radiograph demonstrating the visibility of the radiopaque indicator pin and the reference scale through the stainless-steel tibial plate (110 kV X-ray tube voltage).



Photographs and radiographs of sensor attached to tibial plate on a Sawbones[®] composite bone mimic. Indicator pin position relative to the reference scale demonstrates the poly (AAc-co-n-OA) hydrogel response to pH changes. The pH of the solution was measured at equilibrium after three washes and again after six total washes.

Table ST1

Variable in the sensor	Time constant, τ from the dynamics curve (min)			
Hydrogel thickness (h_i) (mm)	Deswelling-1	Swelling-1	Deswelling-2	Swelling-2
(swollen in pH \approx 7.4)	in pH ≈ 4.0	in pH ≈ 7.4	in pH ≈ 4.0	in pH ≈ 7.4
1.0	29	27	29	24
0.7	23	20	24	16

Effect of hydrogel film thickness on response rate (time constant).