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Top-Down and Bottom-Up Fabrication Techniques for Hydrogel Based Sensing and Hormone Delivery Microdevices

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***Abstract*—We review a set of studies dealing with molecular (glucose) sensing and hormone delivery, in which the swelling and shrinking of a hydrogel as a function of glucose concentration play a central role. Confining hydrogels in microfabricated structures permits transduction of their chemomechanical behaviors. Prototype microdevices for wireless glucose sensing and closed loop insulin delivery control have been designed using hydrogels containing phenylboronic acid sidechains. While these devices exhibit desired responses, improved response time is needed, warranting further miniaturization. In a separate application, geometric confinement of glucose oxidase by a pH-sensitive hydrogel membrane sets up a nonlinear feedback loop which enables rhythmic swell/shrink cycles when the system is exposed to a constant glucose concentration. The latter system may be applied to delivery of gonadotropin release hormone, for which rhythmicity of secretion is essential for therapeutic function.**

I. Introduction

Hydrogels are crosslinked polymer networks that absorb substantial amounts of water. Because of their high water content, hydrogels are excellent models for biological tissues, and they often exhibit excellent biocompatibility. Because the swelling state of a hydrogel is determined by a balance of elastic, polymer-water interaction, and ion osmotic forces, swelling state can be modulated by external variables, or stimuli that impinge on one or more of those forces. Under free swelling conditions, change in swelling may be accompanied by changes in permeability to various solutes. When the hydrogel is mechanically confined, swelling and shrinking cause it to exert stresses on the confining structures, leading to deformations when the latter are sufficiently compliant.

While free swelling is the easiest method to study hydrogel properties, free swelling systems have limited applicability, especially for long-term implantable sensing and drug delivery. By itself, a hydrogel is a soft material with limited storage capacity, relatively weak mechanical strength (tendency to fracture), and susceptibility to attack by host tissue. As will be shown in section II, these problems may be circumvented by placing the hydrogel in chip-like devices formed by top-down micromachining, and using the hydrogel as a mediator for transduction within a more robust device.

In order for a hydrogel embedded in a chip to respond to environmental changes, it must communicate with the environment through pores or channels. Design of the porous interface must take into account rapid exchange of small solutes, exclusion of potential contaminants, and ability to accommodate local tissue response. An effort towards such design is described in section III. In this case, top-down machining of solid micropores is coupled to bottom-up self assembly of block-copolymer nanopores.

Incorporating the hydrogel into a larger structure may also permit new dynamical modes. In section IV an example is presented in which a particular configuration in a device of the hydrogel and the enzyme glucose oxidase leads, via a negative feedback instability, to sustained oscillations in hydrogel swelling and permeability. These oscillations can be harnessed to produce rhythmic delivery of gonadotropin hormone releasing hormone (GnRH), mimicking the natural endogenous pattern of secretion.

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II. Glucose Sensor and Closed-loop Insulin Delivery

A. Hydrogel Chemistry and Function

The earliest attempts to incorporate glucose sensitivity into hydrogels utilized glucose oxidase, which catalyzes conversion of glucose to hydrogen ion (H^+), leading to a local decrease in pH [1-3]. The latter can then react with acidic (carboxylic) or basic (amine) groups on the hydrogel sidechains, according to the general schemes $\text{-}COO^- + H^+ \leftrightarrow \text{-}COOH$ or $\text{-}N + H^+ \leftrightarrow \text{-}NH^+$, respectively. Typically, enhanced ionization of the hydrogel leads to increased swelling. Due to complications associated with long-term enzyme stability, and relative inefficiency of transduction due to buffering of pH by physiological fluids, this enzyme-acid mediated strategy has largely fallen from favor as a means to effect changes in hydrogel swelling.

In the past decade, numerous groups have investigated

hydrogels based on phenylboronic acids (PBAs) [4-7]. As depicted in Fig. 1, diols such as glucose react reversibly with the charged, OH^- complexed form of PBA and stabilize that form, and the fraction, f, of the PBA units that are ionized at a given pH (say physiologic pH=7.4) increases with glucose concentration, C_g, in a roughly Langmuirian manner, i.e. $f = C_g /(K + C_g)$, where K is the apparent dissociation constant at the specified pH.

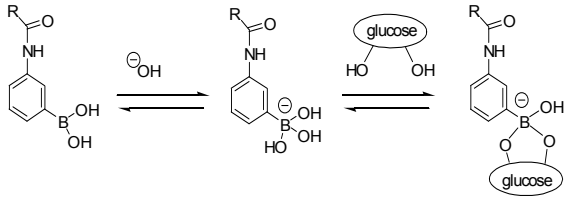

Fig. 1. Scheme by which glucose stabilizes OH^- complexed form of PBA. After Kataoka et al. [6].

Since uncharged PBA's are hydrophobic, they must be incorporated into crosslinked networks containing other, hydrophilic components. In the present studies, hydrogels were formed from copolymers of methacrylamido phenylboronic acid (MPBA: 20 mol%) and acrylamide (AAm: 80 mol%), lightly crosslinked with methylene bisacrylamide (BIS). As shown in Fig. 2, these hydrogels swell to equilibrium according to glucose concentration, and it is easy to discriminate between hypo-, normo-, and hyperglycemia based simply on hydrogel volume.

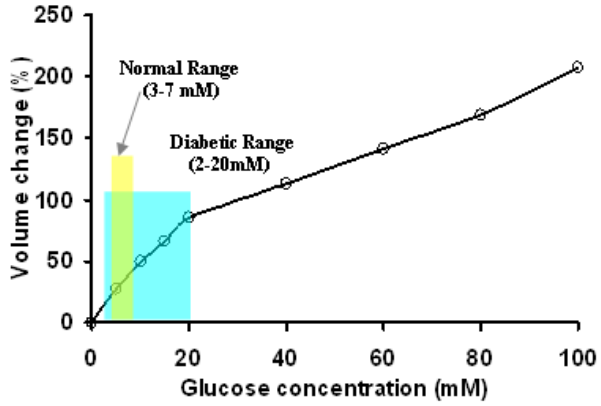

Fig. 2. Relation between equilibrium swelling volume and glucose concentration at pH 7.4, for MPBA/AAm hydrogels.

B. Implantable Wireless Glucose Sensor

While Fig. 2 suggests that free swelling volume can be used to determine glucose concentration, it is exceedingly difficult to carry out this measurement in an implant. A scheme for a wireless, implantable PBA-hydrogel based microsensor, in which the hydrogel is confined between a rigid porous membrane and the top plate of a hermetically sealed microcapacitor, is illustrated in Fig. 3 [8]. The microcapacitor is integrated with a microinductor coil, forming a simple LC resonator. Glucose-dependent swelling pressure exerted by the confined hydrogel displaces the top capacitor plate, increasing the capacitance and reducing the resonant frequency, $1/2\pi(LC)^{1/2}$. This resonant frequency can be detected readily by measuring the phase dip of the impedance to RF stimulation.

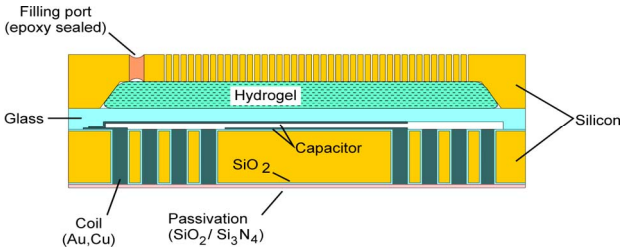

Fig. 3. Schematic of wireless, implantable glucose sensor based on a glucose-sensitive hydrogel confined between a rigid porous membrane (top) and a more compliant, glass diaphragm that is sputter-coated with a metallic layer, forming the top plate of a capacitor in a micro-LC circuit. Reproduced from Ref. [8] with permission.

The microcircuit's quality factor is sufficiently high that adequate resolution of glucose concentration is achieved at equilibrium, as shown in Fig. 4a. However, Fig. 4b shows that the response time of the glucose microsensor is relatively slow (~80 min). In the present system, the hydrogel is ~200 μm thick. Since response is believed to be diffusion limited, it is expected that response time will be proportional to the square of thicknesses of the hydrogel and the rigid membrane. Thus, fourfold reduction in these dimensions is expected to lower response time to ~5min, which is comparable to present glucose sensors.

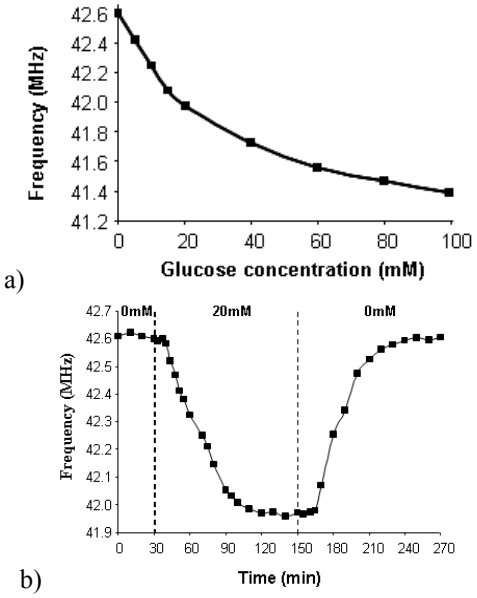

Fig. 4. a) Resonant frequency at equilibrium upon exposure of sensor to glucose at various concentrations, at pH 7.4. b) Response kinetics. Adapted from Ref. [8] with permission.

C. Implantable Glucose-Controlled Insulin Microvalve

The previously described hydrogel-mediated transduction between glucose concentration and mechanical displacement of a microfabricated element is readily adapted to flow control. By replacing the slightly compliant capacitor plate

in Fig. 3 with a flexible silicone rubber diaphragm, and the capacitor gap by a flow channel, with a solid embossment leading from the diaphragm to the vicinity of the channel inlet, a simple microvalve is achieved, as illustrated in Fig. 5 [9]. Hydrogel swelling shuts off the valve, while hydrogel shrinking opens the valve. In principle, this glucose controlled microvalve could be attached to the tip of a transcutaneous catheter leading to an externally worn pressurized aqueous insulin reservoir.

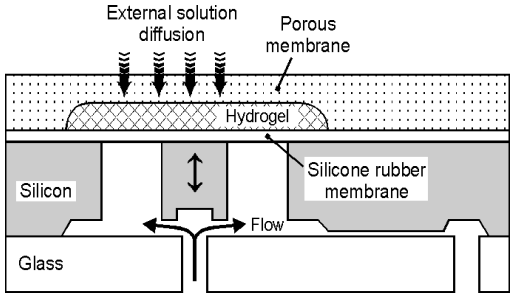

Fig. 5. Schematic of hydrogel mediated glucose controlled microvalve. Adapted from Ref. [9] with permission.

A simple test of this system was carried out by hooking the inlet and outlet fluid ports to tubing, with inlet tubing at 60mm head pressure. Flow was monitored as a function of time, with the valve alternated between two physiologic PBS solutions with glucose concentrations 0 mM and 20 mM. As shown in Fig. 6, this device was able to respond to glucose changes within about 20 min. Unfortunately, the response polarity is the opposite from what is desired—increase in glucose concentration leads to suppressed flow. This problem may be solved either by modifying the mechanics by which hydrogel swelling is transduced into valve opening/shutting, or by incorporating hydrogels that shrink with increasing concentration of glucose due to glucose-mediated crosslink formation [4-7].

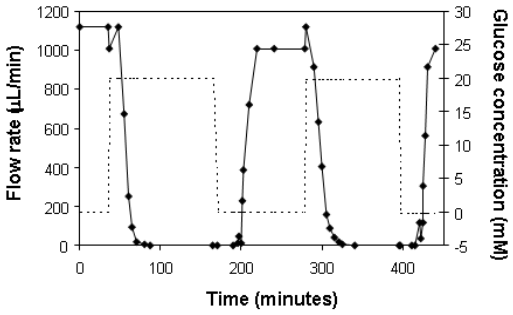

Fig. 6. Schematic of hydrogel mediated glucose controlled microvalve. Adapted from Ref. [9] with permission.

III. Towards Thin But Robust Membranes

In the previous sections, a rigid permeable membrane was used to mediate solute and fluid transfer between the external medium and the confined hydrogel. In order to accelerate response, both the hydrogel and the membrane need to be reduced in thickness. The membrane must remain physically robust, however, and it should provide a favorable interface with the host tissue. Toward these ends, we have constructed an asymmetric platform membrane, consisting of a very thin (~80nm) nanoporous membrane covering a microporous silicon support structure [10].

To produce this membrane, a block polymer consisting of polystyrene-*b*-polyisoprene-*b*-polylactic acid (PS-PI-PLA) was spun onto one side of a silicon nitride coated silicon wafer. Block lengths were chosen such that the polymer self-assembled into a hexagonal array, with PLA forming cylinders in a PS continuum, and PI forming a rim between the PLA and PS. Exposure to NaOH etched away the PLA cylinders, leaving behind PI-lined nanopores with diameters averaging 43 nm ± 11% RSD. Arrays of 20 µm× 20 µm micropores were created in the wafer by a series of dry and wet etches, the sequence chosen so as not to harm the attached nanoporous membrane. Images of the microporous support are shown in Fig. 7a, and a tapping mode AFM height image of the nanoporous membrane, demonstrating its essentially hexagonal character, is shown in Fig. 7b.

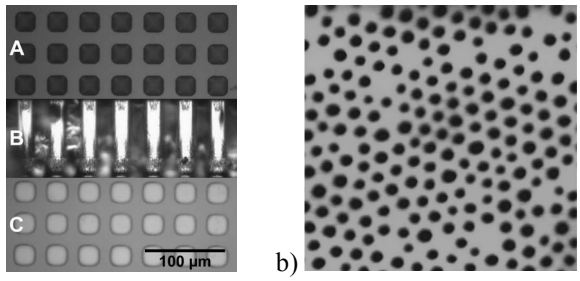

Fig. 7. a) Top (A), cross section (B) and bottom (C) of microporous support membrane. b) Tapping AFM height image of etched nanoporous block polymer membrane. Reproduced from Ref. [10] with permission.

The composite membrane structure is permeable to small molecules, and blocks transport of large molecules such as dextrans with MW $2x10^6$. The PI lining on the nanopore walls permits functionalization with other polymers that may enhance transport selectivity and/or biocompatibility.

IV. Rhythmic Hormone Delivery

Glucose sensing and closed loop insulin delivery provide a canonical example for time-dependent therapeutics. Circadian rhythms have also been studied widely, and some drug administration protocols have been designed with these rhythms in mind [11]. In recent decades, it has been recognized that numerous hormones are secreted in an episodic manner [12, 13]. In particular, GnRH, the "master hormone" driving reproductive development and function, is secreted in rhythmic pulses in both males and females [14].

To emulate the endogenous system, we designed a biochemomechanical oscillator that consumes glucose, presented at *constant concentration* [15, 16], which differs from the *glucose concentration sensitive* systems described

above. This oscillator, depicted schematically in Fig. 8, consists of a chamber containing GnRH and glucose oxidase. The chamber communicates with the constant glucose environment through a hydrogel membrane consisting of the comonomers n-isopropylacrylamide and methacrylic acid. This polyacid hydrogel swells at high pH but collapses at low pH, and the swelling transition exhibits hysteresis. Starting in the swollen state, the membrane permits passage of glucose, which is rapidly converted by the enzyme to H^+, lowering intrachamber pH. As a result, the membrane collapses, blocking further ingress of glucose. Eventually, the produced H^+ diffuses into the external environment, resetting internal pH and bringing the membrane back to its swollen, permeable state. This cycle can repeat indefinitely, in principle, and GnRH release from the chamber will pulsate in synchrony with phases of high membrane permeability. The ability of this device to produce sustained rhythmic release behavior is demonstrated in Fig. 9.

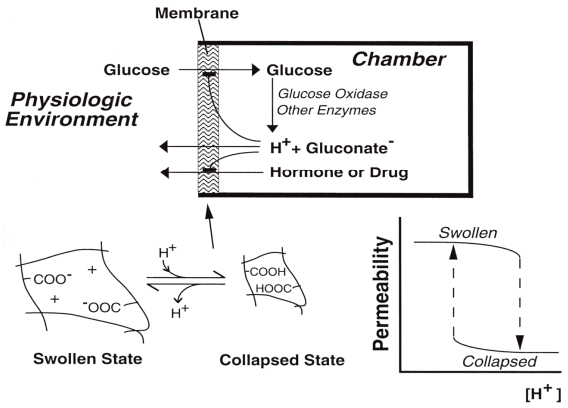

Fig. 8. Schematic of hormone delivery oscillator. Adapted from Ref. [15] with permission.

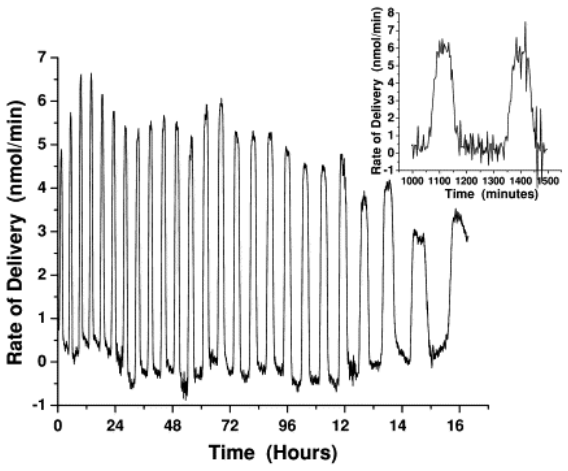

Fig. 9. Rhythmic release (raw data smoothed) of GnRH from device schematized in Fig. 8. Inset shows detail of unsmoothed 5th and 6th peaks. Reproduced from Ref. [16] with permission.

V. Conclusion

While free swelling of hydrogels is a convenient means to characterize their response to various stimuli, free hydrogels are rarely useful as devices. As discussed in this paper, swelling response can be put to use in sensing and drug delivery by integrating hydrogels with hard and soft materials produced by top-down and bottom-up techniques. Complex behaviors such as autonomous oscillations can be observed by constraining the spatial organization of a hydrogel with respect to an enzyme. Future applications of stimuli sensitive hydrogels will involve other kinds of integration with micro- and nanofabricated systems.

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