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Mechanistic studies of an autonomously pulsing hydrogel/enzyme system for rhythmic hormone delivery

Amardeep S. Bhalla [a], Ronald A. Siegel [a,b,*]

[a] Department of Pharmaceutics, University of Minnesota, Minneapolis, MN 55455, USA
[b] Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455, USA

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ABSTRACT

Numerous hormones are known to be endogenously secreted in a pulsatile manner. In particular, gonadotropin replacing hormone (GnRH) is released in rhythmic pulses, and disruption of this rhythm is associated with pathologies of reproduction and sexual development. In an effort to develop an implantable, rhythmic delivery system, a scheme has been demonstrated involving a negative feedback instability between a pH-sensitive membrane and enzymes that convert endogenous glucose to hydrogen ion. A bench prototype system based on this scheme was previously shown to produce near rhythmic oscillations in internal pH and in GnRH delivery over a period of one week. In the present work, a systematic study of conditions permitting such oscillations is presented, along with a study of factors causing period of oscillations to increase with time and ultimately cease. Membrane composition, glucose concentration, and surface area of marble ($CaCO_3$), which is incorporated as a reactant, were found to affect the capacity of the system to oscillate, and the pH range over which oscillations occur. Accumulation of gluconate$^-$ and Ca^{2+} in the system over time correlated with lengthening of oscillation period, and possibly with cessation of oscillations. Enzyme degradation may also be a factor. These studies provide the groundwork for future improvements in device design.

1. Introduction

Numerous hormones are endogenously secreted in a pulsatile, episodic manner, the periodicity of which is as important as the chemical structure of the hormone [1–4]. Rhythmicity or quasirhythmicity of hormones often occurs in an ultradian fashion, i.e. several cycles per day. In some cases, periodicity of secretion is believed to be set to match the kinetics of desensitization and resensitization of target receptors [1,5].

For example, gonadotropin releasing hormone (GnRH: aka luteinizing hormone releasing hormone, LHRH) is a decapeptide "master hormone" that is released rhythmically from the hypothalamus every 1–2 h under neuroendocrine control [6–8]. Following portal transport to the anterior pituitary gland, GnRH stimulates hypophysial secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH). These peptide hormones travel to the gonads, where they stimulate secretion of other peptide and steroid sex hormones such as secretin, estradiol, testosterone and progesterone. Insufficient secretion of GnRH results in reproductive disorders associated with hypogonadotropic hypogonadism (HH), which occurs in both men and women, and is manifested in arrested or regressed sexual maturation and fertility [4,9]. Treatment of hormonal disorders associated with HH warrants hormone delivery in a temporal pattern mimicking the endogenous ultradian rhythm. Studies have demonstrated the ability of pulsed GnRH to restore reproductive function in women with HH, while continuously administered GnRH and its synthetic analogs suppress reproductive function [10].

Several approaches to ultradian GnRH delivery have been proposed [11–14]. Wearable intravenous and subcutaneous pumps with rhythmic external control have an established track record for GnRH fertility therapy, but these devices carry risk of infection and other inconveniences. Transdermal iontophoretic delivery of GnRH has been investigated [15,16], as have been the intranasal and buccal routes [17,18]. To date, however, no adequate substitute for pumps has advanced.

The high potency and hence extremely low dose requirement of hormones such as GnRH suggest that implanted systems could provide ultradian doses over extended time periods. It has been proposed, for example, that small doses of GnRH be released from microfabricated multi-well chips, with different wells activated in sequence by electrodissolution or electrothermal destruction of sealing layers [19,20]. Such systems require electrical power provided either by an external source with transcutaneous wires, or a co-implanted battery.

Previous work demonstrated the principle of the autonomous rhythmic hormone delivery system based on a hydrogel and enzymes [21,22], described in Section 2. Under proper conditions, the system exhibits ultradian oscillations in internal pH and coherent pulses of released GnRH. However, it was also found that these rhythmic behaviors,

* Corresponding author at: Pharmaceutics 9-177 WDH, University of Minnesota, 308 Harvard St SE, Minneapolis, MN 55455, USA. Tel.: +1 612 624 6164; fax: +1 612 626 2125.
E-mail address: siege017@umn.edu (R.A. Siegel).

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when present, are not sustained indefinitely. The period of pH oscillation, and hence the interval between pulses of GnRH release, increases with time, and eventually oscillations cease.

The ability of the system to produce pH oscillations and rhythmically pulsed GnRH release depends critically on membrane chemistry and other design features, and on glucose concentration. The present study deals with effects of certain design parameters on the system's capacity to oscillate, with factors causing progressive increase in period of pH oscillations, and with factors that lead to the ultimate cessation of those oscillations. Due to the expense of working with GnRH, the hormone was not included in these studies. It is presumed that pulsatile GnRH release will correlate with pH oscillations as shown before—this assumption will have to be reconfirmed at further development stages. Since GnRH is highly potent and can be present in very low concentrations, it is not expected to perturb the system's dynamics.

Section 2 provides a brief review of the hydrogel/enzyme based oscillator and experiments that have been previously reported. Experimental methods for the present work are described in Section 3. In the first part of Section 4, the effects of system parameters on the capacity of the system to initiate sustained oscillations are studied. In the second part of Section 4, factors contributing to lengthening of oscillation period and ultimate cessation of oscillations are investigated. Discussion, conclusions and suggestions for future work are presented in Section 5 and 6.

2. General background

The ultradian rhythmic hormone delivery system under investigation consists of a chamber containing *GluOx*, *Cat* and *GluLac*, plus the hormone to be released (e.g. GnRH). The chamber communicates with the external environment, which contains a constant concentration of glucose, through a poly(*N*-isopropylacrylamide-*co*-methylacrylic acid) [poly(NIPAM-*co*-MAA)] hydrogel membrane. This membrane exhibits a sharp transition in permeability to glucose with change in pH inside the chamber. When the membrane is in its charged and swollen state (MAA sidechains deprotonated), glucose diffuses from outside through the membrane into the chamber, where it is rapidly converted to hydrogen ions according to the reactions

$$\begin{aligned} &\text{Glucose} + O_2 + H_2O \xrightarrow{GluOx} \text{Glucono lactone} + H_2O_2 \\ &\text{Glucono lactone} \xrightarrow{GluLac} \text{Gluconic Acid} \rightarrow \text{Gluconate}^- + H^+ \\ &H_2O_2 \xrightarrow{Cat} \tfrac{1}{2}O_2 + H_2O. \end{aligned} \quad \text{(I)}$$

The first reaction produces gluconolactone and H_2O_2, an unwanted byproduct [23,24]. The second reaction converts gluconolactone to gluconic acid, which rapidly dissociates to gluconate$^-$ and H^+ (pKa = 3.76, well below the operating pH range of the oscillator), decreasing intrachamber pH. The third reaction eliminates H_2O_2 and restores half of the O_2, which is then available to participate in the first reaction. In the studies conducted here, enzymes were added in excess, so that the reactions were fast compared to other processes, even away from their optimal pH ranges.

Following reactions (I), hydrogen ions diffuse from the chamber into the membrane and neutralize the charged MAA groups, eventually leading to collapse of the membrane and attenuation of glucose influx. Consequently, hydrogen ion production inside the chamber is reduced, and intrachamber pH increases. Subsequent loss of acidic protons from the membrane into the external medium and reswelling of the membrane reestablish glucose permeability, and conditions are now in place to repeat the cycle. The cyclic changes in intrachamber pH, membrane charge, and membrane swelling underlie the rhythmic release of hormone across the membrane.

Initial studies established that poly(NIPAM-*co*-MAA) hydrogel membranes, exposed on one side to constant pH 7.0 but with the other side exposed to media of varying pH values, exhibit large transitions in permeability to glucose as a result of collapse and swelling of hydrogel proximal to the variable pH side [25,26]. The collapse and swelling transitions occur at different pH values on the variable side, and the membrane's permeability therefore exhibits bistability and hysteresis in the pH range between those values. This property is very useful in promoting oscillations. Without it, the membrane would likely relax to an intermediate, stationary swelling/permeability state in which glucose influx, enzymatic conversion, and clearance of H^+ are in exact balance, with no oscillations.

Later studies showed that the collapsed, impermeable state of the hydrogel membrane is temporally unstable. While a pH drop leads initially to collapse and near shutoff of permeation, eventually the membrane reverts to a state of intermediate permeability, which appears to arise by accumulation of lateral stress on the collapsed side, followed by phase separation into collapsed and partially permeable swollen domains [27]. The importance of this observation will become apparent below.

Fig. 1 is a schematic of a bench prototype of the oscillating hormone delivery system. The prototype is a side-by-side diffusion apparatus, with the poly(NIPAM-*co*-MAA) separating two well stirred cells. Cell I corresponds to the external environment. Aqueous glucose solution flows into Cell I at a fixed rate, and waste is removed at an equal flow rate. Cell I is maintained at pH 7.0 by a pH stat. Cell II, which corresponds to the rhythmic delivery device's internal chamber, contains the enzymes. Fluctuations of pH in Cell II are monitored by an electrode. Details are provided in Section 2.

The earliest experiments with this prototype yielded very slow, transient changes in pH in Cell II, but no sustained oscillations, probably due to the appearance of the proposed intermediate permeability state of the membrane [26]. It was hypothesized that this intermediate state is a trap into which the system can fall if pH changes are not sufficiently rapid [27]. To ensure rapid pH changes in Cell II, external glucose concentration was increased and granular marble was introduced into Cell II. Heterogeneous reaction of H^+ with marble ($CaCO_3$) provides a shunt pathway for H^+ clearance via the reactions [28].

$$\begin{aligned} &H^+ + CaCO_3(s) \rightarrow Ca^{2+} + HCO_3^- \\ &H^+ + HCO_3^- \leftrightarrow CO_2 + H_2O. \end{aligned} \quad \text{(II)}$$

Combining the enhanced rate of H^+ production due to increasing glucose flux across the membrane, and enhanced rate of H^+ depletion due to the marble reaction, the up and down slew rates of pH in Cell II are increased. With proper adjustments, transitions between the high and low permeability states of the hydrogel membrane occur with sufficient rapidity that the membrane does not fall into the intermediate permeability trap. With these modifications, pH in Cell II exhibits sustained ultradian oscillations, with concomitant rhythmic pulses of GnRH release across the membrane [22,29]. With increasing duration

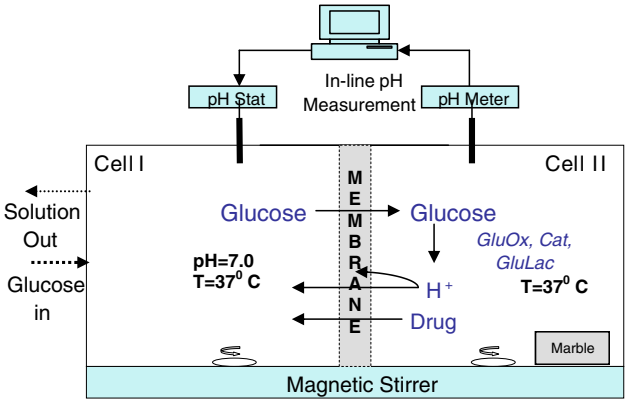

Fig. 1. Schematic of glucose driven chemomechanical oscillator.