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Manuel Ochoa
Birck Nanotechnology Center, Purdue University, ochoam@purdue.edu

Babak Ziaie
Birck Nanotechnology Center, Purdue University, bziaie@purdue.edu

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A fermentation-powered thermopneumatic pump for biomedical applications†

Manuel Ochoaabc and Babak Ziaieabc

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We present a microorganism-powered thermopneumatic pump that utilizes temperature-dependent slow-kinetics gas (carbon dioxide) generating fermentation of yeast as a pressure source. The pump consists of stacked layers of polydimethylsiloxane (PDMS) and a silicon substrate that form a drug reservoir, and a yeast-solution-filled working chamber. The pump operates by the displacement of a drug due to the generation of gas produced via yeast fermentation carried out at skin temperatures. The robustness of yeast allows for long shelf life under extreme environmental conditions (50 °C, >250 MPa, 5–8% humidity). The generation of carbon dioxide is a linear function of time for a given temperature, thus allowing for a controlled volume displacement. A polymeric prototype (dimensions 15 mm × 15 mm × 10 mm) with a slow flow rate of <0.23 µL min⁻¹ and maximum backpressure of 5.86 kPa capable of continuously pumping for over two hours is presented and characterized.

Introduction

Autonomous micropumps capable of delivering fluids at a low flow rate over an extended time (several hours) without any electrical power source are sought after for drug delivery and other lab-on-a-chip (LOC) applications. For example, although transdermal drug delivery via microneedle arrays has long been identified as a viable and promising method for delivering large hydrophilic molecules across the skin, a suitable pump that can be used with such systems has been hard to attain. Most pumps proposed for drug delivery applications rely on an on-board power source which is bulky, imposing space/cost limitations, and making power management a key design feature. For example, among the commercialized systems for drug delivery (e.g., insulin), the OmniPod® pump (a stepper motor pushing a plunger, http://www.myomnipod.com) is capable of sustaining a flow rate of 500 nL min⁻¹ but requires four button batteries and is over 40 cm³ in total volume (pump plus batteries and other electronics). Even much smaller/microfabricated commercialized pumps such as Debiotech’s Nanopump® (piezoelectrically actuated, delivering 150 nL per actuation cycle to achieve flow rates in the hundreds of nanoliters per minute, http://www.debiotech.com) require high driving voltages (110 V peak) and on board power conditioning circuitry to convert a DC battery voltage to a high voltage pulse sequence. Osmotic micropumps capable of flow rates in the nL–µL per minute do not require a power source and provide their own driving force via osmotic pressure gradient. Such pumps, however, require an aqueous surrounding for their operation, thus making them unsuitable for many extracorporeal applications. Alternative power sources that rely on localized biological or chemical reactions (e.g. responsive hydrogel systems) present a more favorable approach to provide energy for autonomous micropumps. Our group recently demonstrated a body heat actuated pump using phase-change high vapor pressure fluorocarbon liquids to provide bolus injection across the skin. While the battery-less operation was advantageous, the high volatility of fluorocarbon liquids and its potential evaporation through materials such as PDMS poses a restriction on the device shelf life and fabrication materials.

In this paper, we describe a miniature pump (Fig. 1a) that utilizes the process of fermentation of glucose by yeast to provide a pneumatic driving force (in the form of carbon dioxide gas) for a low flow rate, long-term dispensing system. The robustness of dry yeast allows for a long shelf life (50 °C, >250 MPa, 5–8% humidity), and its reliable fermentation process offers sufficient pneumatic energy to power a pump. Dry yeast cells can remain dormant for years and be easily activated by the addition of a warm aqueous sugar solution. This results in devices that have a long shelf life with no need for refrigeration and can be activated simply by the addition of water and contact with body heat. Alternatively, due to the strong temperature-dependent response of yeast, the pumps may be loaded with a prepared liquid solution of yeast-glucose and stored for days at common refrigerator temperatures without activating the yeast.

In S. cerevisiae yeast used in this work, glucose fermentation proceeds anaerobically by the following reaction via a naturally occurring yeast enzyme complex, zymase.

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A sucrose solution can alternatively be used; in this case, the sucrose is first broken down into glucose by a naturally occurring invertase enzyme via the following reaction:

\[ C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{invertase}} 2C_6H_{12}O_6 \]

The yeast cells will continue to ferment until resources are depleted or the alcohol concentration reaches a toxic level (typically 15–18% by volume for commercial active dry yeast). The amount of glucose that needs to be fermented to reach this level is much greater than the amounts implemented in this paper; hence, cells remain fully viable throughout the operation of the device. As mentioned above, the fermentation process is strongly temperature-dependent and pumping action can be initiated by a simple skin-contact (e.g., for micropumps used in transdermal drug delivery). The species of yeast employed in this pump (S. cerevisiae) has been shown to have an optimal metabolic activity close to body temperature \(25^\circ C\) and to withstand high hydrostatic pressures (>250 MPa). The fermentation process has slow kinetics resulting in a controllable dispensing rate of a few tenths of a microliter per minute or less for an extended time period (>2 h at constant flow rate, maximum of 10 h for our device).

**Materials and methods**

Solutions of yeast were prepared at room temperature by adding various concentrations (0.75 g L\(^{-1}\), 1.5 g L\(^{-1}\), 3 g L\(^{-1}\), 6 g L\(^{-1}\)) of Fleischmann’s \(^R\) Active Dry \(^R\) yeast cells to a 0.10 M aqueous solution of glucose. The mixture was stirred to induce homogeneity and to activate the yeast. The solutions were subsequently injected into the devices for testing.

The rate of gas generation by the various preparations of yeast solution was measured by a pneumatic trough setup to assess the linearity over time. For each yeast preparation (concentration), a 50 mL sample of the solution was placed in a flask and heated to \(32^\circ C\) on a hotplate. The flask was connected to a graduated cylinder previously filled with water so that any gas generated in the flask would displace water in the cylinder. Water displacement was measured over time to assess the generation rate of the working gas.

The drug-dispensing pump described here (Fig. 1a) was fabricated from four PDMS (Sylgard 184, Dow Corning, Auburn, MI) layers and a silicon substrate. It consists of two chambers separated by an elastic membrane. One of the chambers (the drug chamber, 2 mm height \(\times\) 8 mm diameter, 100 \(\mu\)L in volume) is capped at one end by the membrane (140 \(\mu\)m thick) and at the other by a thick (2 mm) wall containing an output port with one or more needles, whereas the other chamber (the pressure chamber, 2 mm height \(\times\) 8 mm diameter, 100 \(\mu\)L in volume) is capped at one end by the membrane and at the other end by a rigid substrate of a thermally conductive material (i.e. 500 \(\mu\)m thick silicon). All layers were bonded together via plasma-induced surface modification. Fig. 1b shows the cross-sectional photograph of a fabricated pump illustrating various reservoirs and layers.

For characterization, the devices were first sterilized in an oven at 131 °C for 30 min and cooled down to room temperature.
temperature. Subsequently, the yeast and drug chambers were filled with a hypodermic needle syringe, making sure to vent the previously trapped air from the chambers while filling. A graduated capillary tube (400 µm in diameter) was connected at the drug outlet of each device to allow for the measurement of flow rate. The devices were tested at different temperatures on a hotplate (25 °C, 30 °C, 32 °C, 34 °C), with temperature controlled by feedback from a thermal probe attached to the hotplate surface (samples were also tested at 0.6 °C in a refrigerator). The volume of dispensed liquid from the drug chamber was measured visually over time to determine the flow rate.

Results and discussion

Fig. 2 shows the experimental results of the gas generation for various yeast concentrations at a constant temperature of 32 °C. The data show that the amount of generated gas is a linear function of time for at least 2 h. Additionally, an extended time test revealed a constant rate of gas generation for up to 10 h, with operation continuing non-linearly for up to 20 h (Fig. 3). As is typical of microorganism cultures, the growth rate of yeast can be modeled by the logistic equation. During the period before retarding processes become influential, the number of cells can be modeled by an exponential function of time and during much shorter periods (within the time range of the presented pumps), population is essentially linear with time. A theoretical estimate can be carried out to verify this assumption. If the yeast population size is assumed to be \( n(t) = n_0 e^{kt} \) (\( n_0 \) is the initial cell count in the solution, \( \sim 20 \) billion per gram of dry yeast, and \( k \) is the growth-rate time constant) and each cell is assumed to ferment \( F \) grams of glucose per unit time, then the total amount (grams) of fermented glucose is given by

\[
\int_0^t n(t) F \, dt = \frac{2n_0 F}{k} \left( e^{kt} - 1 \right)
\]

Using stoichiometric ratios and assuming ideal gas conditions at standard temperature and pressure, the volume of generated CO\(_2\) gas can be represented by

\[
\frac{2n_0 F V_m}{M_k} \left( e^{kt} - 1 \right)
\]

where \( M \) is the molar mass of glucose and \( V_m \) is the molar volume of the gas (22.4 L mol\(^{-1}\)). By a similar calculation, the volume of ethanol generated can be modeled by

\[
\frac{2n_0 F \rho}{M_{EOH} k} \left( e^{kt} - 1 \right)
\]

where \( M_{EOH} \) is the molar mass of ethanol and \( \rho \) is its density.

The gas generation measurements were used to deduce an approximate rate of glucose generation (\( F \)) and yeast growth rate constant (\( k \)) for the strain of active dry yeast used in the glucose solution. The yeast cultures exhibited a growth rate constant of \( k \approx 3.67 \times 10^{-3} \text{ min}^{-1} \); with this constant, a fermentation rate of \( F \approx 4.95 \times 10^{-13} \text{ g cell}^{-1} \text{ min}^{-1} \) can be deduced from the data. The calculated ethanol concentration, Fig. 3, is low enough (<15% by volume) during the first 10 h of operation that it will not severely impact the rate of fermentation. An extrapolation of the calculated gas generation over time (plot available in the ESI\(^+\)) revealed a linear trend for up to 3 h for the lower concentrations of yeast (<3 g L\(^{-1}\)). Such a linear response makes the yeast solution a very useful and scalable gas source for autonomous micropump applications, since the flow-rate of the device at a given temperature can be controlled simply by the concentration of yeast in the device.

Fig. 4a shows the output volume vs. time for the fermentation-actuated pumps at various temperatures. An average flow rate of
0.23 μL min⁻¹ over 120 min was obtained for 100 μL of (3 g L⁻¹) yeast solution at 32 °C. The dispensing rate is highest for temperatures within the optimum range of yeast metabolic activity (32–35 °C) and decreases at lower temperatures. The small difference in flow rate between the samples tested at 32 °C and 34 °C show a tolerance for temperature variations in the optimum temperature range. At the low temperature end, flow rates are essentially negligible, as is expected due to the slowed metabolic activity of yeast. The slightly non-linear response during the first few minutes of operation can be attributed to device parameters rather than fermentation kinetics. In particular, the elastic membrane between the actuation and drug chambers is a major cause of this non-linear behavior due to imperfections in its tautness at very low pressures. Additionally, the permeability²⁶ of PDMS to CO₂ may contribute to the irregularities. The use of PDMS as the primary fabrication
material was intended as a proof of concept for the pump; future devices can utilize a thin coat of a gas-impermeable material (e.g. parylene) on the membrane, or, alternatively, they can be fabricated using an elastomer with lower gas permeability.

Fig. 4b shows the flow rate response with an applied backpressure. For these measurements, the pump was connected to a pressure-regulated nitrogen source, and the flow rate was measured in the same manner as above. The results depict a flow rate profile that is inversely proportional to the applied backpressure. This qualitative trend is understandable, since the flow rate is proportional to the molar volume of the gas at the given pressure and the molar volume varies inversely with pressure. A maximum backpressure of 5.86 kPa was obtained for the given pressure and the molar volume varies inversely with the flow rate. This qualitative trend is understandable, since the flow rate is proportional to the molar volume of the gas at the given pressure and the molar volume varies inversely with pressure. The fermentation rate is optimum for temperatures close to the skin temperatures. The robustness of yeast allows for long-term storage in dry form and quick activation with a sugar solution.

Conclusions

In this paper, we presented a skin-contact actuated pump for drug delivery that uses S. cerevisiae yeast fermentation of sugar as its driving mechanism. A multi-hour (2–20 h) sustainable dispensing with an average flow rate of 0.23 μL min⁻¹ over two hours was obtained at 32 °C. The device is particularly suited for applications requiring a low flow rate over an extended period of time. The fermentation rate is optimum for temperatures close to the skin temperatures and the device can tolerate small variations in skin temperatures. The robustness of yeast allows for long-term storage in dry form and quick activation with a sugar solution.

References