Microfluidic Exchange Devices for Cell-free Reactions

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The development of cell-free protein synthesis (CFPS) technology enables creation of a potentially flexible tool for production of protein-based therapeutics in a variety of formats. Although cell-based methods are more established, CFPS systems allow for greater control over the reaction conditions and can be optimized for the production of a desired product. The breadth of cell-free applications is expanded greatly when combined with micro- and nano-scale technologies; however, the focus of this work has been on the development of protein arrays and screening tools. Another exciting adaptation for cell-free reactions in microfluidics is the development of reactors with micro-or milli-liter scale capacities ideal for the on-demand synthesis of single-dose therapeutics.

Yields and concentrations are difficult to optimize in micro-scale systems, which aid reaction speed by containing reactants in small volumes, but also concentrate inhibitory molecules. The ability to dialyze or feed cell-free reactions is necessary to prolong protein production beyond a few hours. To that end, several cell-free micro-scale systems with membrane dialysis capabilities have been engineered[1,2]. Our goal has been to develop a continuous exchange micro-reactor, using a serpentine microchannel device (Figure 1a), capable of producing a single therapeutic dose of a protein. To achieve this goal, the reactor must be compatible with other micro-scale protein purification and processing modules. The serpentine channel design allows for continuous production of protein that may be fed directly into a purification system. The microchannel devices that we have designed allow mixing of up to three components that are fed into a common reactor channel, running parallel to a feeder channel that supplies the reaction with additional energy and metabolites (Figure 1b). The two channels are separated by a nanoporous membrane created using electron-beam lithography. The advantages of these devices include rapid mixing of the components as they flow through the channel and the ability to perform batch or continuous-flow reactions. Additionally, the permeability of the nanoporous membrane can be tuned using different techniques, such as plasma-enhanced chemical vapor deposition and atomic layer deposition (Figure 1c) to optimize exchange and improve yields above those of available commercial technologies (Figure 1d).

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[1] **Khnouf R, Beebe DJ, Fan ZH.** Cell-free protein expression in a microchannel array with passive pumping. *Lab Chip* 9: 56-61, 2009.

[2] **Siuiti P, Retterer ST, Doktycz MJ.** Continuous protein production in nanoporous picolitre volume containers. *Lab Chip* 11: 3523-3529, 2011.

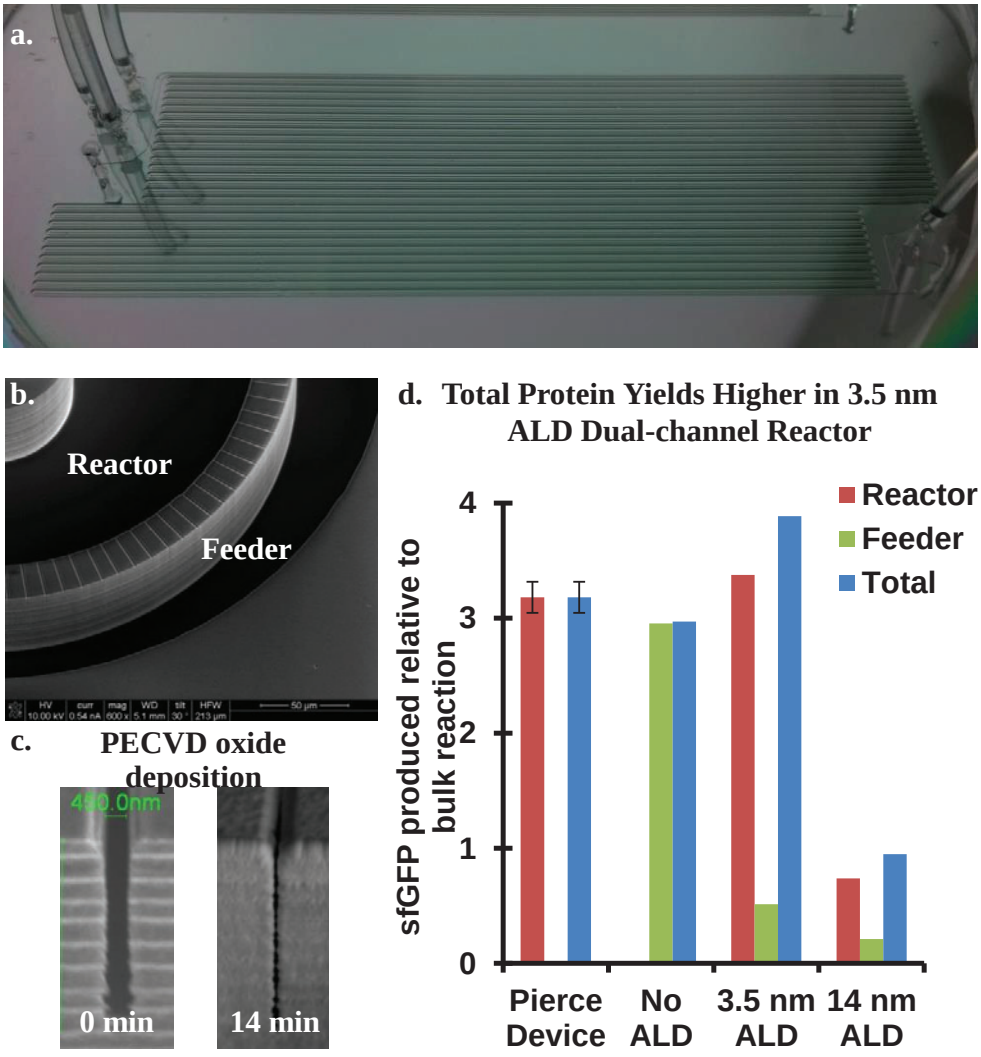

Figure 1: Exchange Reactor Design and Reactor Yields: (a) Dual-channel, exchange reactor made from silicon with a 50 µl micro-channel reactor running in parallel to a feeder channel separated by a wall with nanoslits, and sealed with a PDMS lid. (b) SEM image of one turn of the serpentine dual-channel reactor. (c) The silicon devices are treated with PECVD and ALD to close the membrane. Pictured is an example of the nanoslits before and after a 14 minute PECVD treatment. (d) Preliminary results comparing dual-channel reactors with different porosities to the Pierce microdialysis device as a commercial control. The permeability of the nanoporous membrane in each of the dual-channel reactors was tuned with 14 minutes of PECVD, followed by the addition of more silicon dioxide, as indicated, using atomic layer deposition.