Characterization of enzymatic micromachining for construction of variable cross-section microchannel topologies

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The ability to harness enzymatic activity as an etchant to precisely machine biodegradable substrates introduces new possibilities for microfabrication. This flow-based etching is straightforward to implement, enabling patterning of microchannels with topologies that incorporate variable depth along the cross-sectional dimension. Additionally, unlike conventional small-molecule formulations, the macromolecular nature of enzymatic etchants enables features to be precisely positioned. Here, we introduce a kinetic model to characterize the enzymatic machining process and its localization by co-injection of a macromolecular inhibitor species. Our model captures the interaction between enzyme, inhibitor, and substrate under laminar flow, enabling rational prediction of etched microchannel profiles so that cross-sectional topologies incorporating complex lateral variations in depth can be constructed. We also apply this approach to achieve simultaneous widening of an entire network of microchannels produced in the biodegradable polymeric substrate poly(lactic acid), laying a foundation to construct systems incorporating a broad range of internal cross-sectional dimensions by manipulating the process conditions. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4948508>]

INTRODUCTION

Incredible progress has been made toward adapting microfluidic systems toward a broad range of analytical functions,^{1–4} but these advancements also place increasing demands on microfabrication technology. Conventional approaches continue to be largely based on photolithography methods that are optimally suited for construction of planar topologies with a relatively low complexity and constant cross-sectional dimensions.⁵ These limitations become problematic when applied to construct branched microchannel architectures that mimic physiological systems in nature (e.g., vascular and respiratory networks), where a hierarchy of cross-sectional dimensions is required.⁶ A variety of specialized techniques (e.g., deep reactive ion etching⁷ and laser-induced backside wet etching⁸) can be employed to construct features with variable cross-section, but these methods generally require dedicated instrumentation and complex process flows.

We previously explored flow-based micromachining using a three-component system consisting of an enzymatic etchant (proteinase K; PK), a macromolecular inhibitor (bovine serum albumin, BSA), and a biodegradable substrate (poly(lactic acid), PLA) as a means to overcome these limitations.⁹ We successfully applied this approach to construct a continuous size-based filtration architecture based on a microchannel with different depths on the left- and right-hand

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sides of the cross-section, connected by a narrow weir-like central gap. The gap was sized to selectively permit lateral transport of species below a specified cutoff size between the adjacent deeper regions, enabling selective isolation and enrichment of different sized cellular components from a mixture. Enzymatic micromachining made it possible to imprint this cross-sectional topology in a lithography-free manner, without the need for specialized instruments or facilities, and the use of hazardous toxic etchants that require careful handling and disposal.

In this paper, we build on our previous proof of concept studies by introducing a kinetic model of enzymatic micromachining that enables the key governing parameters to be elucidated so that they can be rationally manipulated to imprint desired topological configurations. We also apply this enzymatic approach to uniformly etch all interior surfaces within a branched microchannel architecture. The ability to simultaneously manipulate the cross-sectional dimensions of an entire network of microchannels introduces the possibility to construct systems incorporating a broad range of geometric parameters using only a single design. Moreover, unlike conventional etching and soft lithography processes, the interplay between enzymatic etchant and macromolecular inhibitor can also be exploited to generate multi-depth microchannels without the need for stacking or alignment steps. These unique capabilities provide a readily accessible toolbox to enable convenient fabrication of topologically complex microfluidic systems.

MATERIALS AND METHODS

PLA etching substrate preparation

Disc-shaped substrates were produced by placing pelletized PLA resin (NatureWorks grade 3051; Jamplast, Inc., Ellisville, MO) inside 1.5-in diameter hollow cylindrical Teflon molds. The cylindrical molds were stacked on a base layer of poly(dimethyl siloxane) (PDMS) to produce a smooth surface of PLA and to enable easy release of PLA from the mold. The mold assembly was heated to 180 °C under vacuum for 1 h. Once the PLA pellets were completely melted, the vacuum was released and heating continued for additional 1 h. The molds were then removed from the oven and cooled at room temperature, after which the solidified PLA substrates were removed from the mold for use in subsequent experiments.

Microfluidic etching template

Microchannel templates for laminar flow etching were fabricated in PDMS using soft lithography.⁵ Briefly, master molds were constructed using SU-8 2050 photoresist (Microchem) applied to a 35 mm silicon wafer by spin coating at 3200 rpm for 60 s, yielding a 50 μ m thick layer. Patterns were photolithographically defined using a mask printed on transparency film at 20320 dpi. The resulting photoresist structures were then used as molds to replicate the microchannel patterns in PDMS (Sylgard 184; Dow Corning) prepared using a 10:1 degassed mixture of prepolymer and curing agent. After curing for 2 h at 80 °C, the cured PDMS was cooled to room temperature and peeled from the mold. Fluidic access ports were punched with a 22 gauge blunt-end needle. The microchannel molds were rinsed with 2-propanol (Fisher Scientific), dried in a stream of compressed air, and directly adhered to the flat surface of a PLA substrate to produce enclosed channel networks. Tygon tubing (0.03 in. O.D.; Small Parts, Inc.) was inserted into the access ports, and the entire assembly was clamped between two acrylic sheets to provide a tight seal.

Enzymatic etching

Aqueous solutions of proteinase K (MW = 28.9 kDa; BP1700, Fisher Scientific) and BSA (MW = 66.4 kDa; A2153, Sigma-Aldrich) were prepared in 30 mM Tris-HCl buffer (pH 8.0, BP1758; Fisher Scientific). An etchant concentration of [PK] = 6.92 μ M (0.2 mg/ml) was used in most experiments, unless otherwise indicated. Prior to etching, the PDMS template was thoroughly rinsed with deionized (DI) water. The PDMS mold and PLA substrate were temporally bonded by pressing them together, and the entire assembly was clamped between two acrylic sheets to ensure a tight seal. While we did not do a quantitative leak test, we observed that

flow rates of at least 100 $\mu\text{l}/\text{min}$ could be applied without leakage (very low flow rates are applied during etching). A syringe pump (PHD 2000; Harvard Apparatus) was connected, and the entire assembly was placed inside an incubator while the etching solutions (PK and BSA) were continuously pumped through the network at 37 °C. After etching was completed, the PDMS template was peeled from the PLA substrate and the resulting patterns were washed using 2-propanol followed by DI water to stop the enzymatic reaction. Etched channel depths were measured using a Dektak 3 stylus profilometer (Veeco Instruments). Surface topologies and cross-section profiles were imaged using a JEOL JSM-6400 scanning electron microscope.

Visualization of flow and etching

Images of the flow field inside the microchannels were obtained using a Zeiss Axiovert 200M fluorescence microscope. Bright field images of co-injected food dye streams were used to visualize the interface under different flow conditions. Diffusive transport of BSA was observed using FITC-BSA (A9771; Sigma-Aldrich). Images were acquired using Zeiss AxioVision Software version 4.8 with a FITC filter set.

Patterned PLA substrate preparation

Negative patterns were photolithographically defined using a mask printed on transparency film at 65 024 dpi. The resulting photoresist structures were then used as master molds to replicate the inverse microchannel patterns in PDMS prepared using a 10:1 degassed mixture of prepolymer and curing agent. After curing for 2 h at 80 °C, the cooled PDMS slab was peeled from the master mold as a soft elastomer template, rinsed with 2-propanol (Fisher Scientific), and dried in a stream of compressed air. Positive replicas of the patterned microchannels were produced in PLA by directly placing pelletized PLA resin onto the PDMS mold and heating to 180 °C for 1 h under vacuum, followed by another 1 h at atmospheric pressure and cooling to room temperature for 1 h. After release from the PDMS mold, fluidic access ports on the PLA microchannel were drilled with a 1/32-in. bit. Smooth planar substrates were obtained by placing pelletized PLA resin directly onto glass microscope slides. A silane pre-treatment was applied to enable easy release of the PLA film by exposing the slides to tridecafluoro-1,1,2,2-tetrahydrooctyltrichlorosilane vapor (SIT8174.0; Gelest, Inc.) inside a desiccator for at least 2 h at room temperature. The glass slides containing the PLA pellets were heated to 180 °C under vacuum for 1 h, followed by an additional 1 h of heating after the vacuum was released. The slides were then cooled at room temperature for 1 h, after which the PLA film could be easily peeled away. Both the patterned microchannel and flat PLA plates were rinsed with 2-propanol followed by DI water and dried with compressed air. Enclosed microchannels were fabricated by directly placing patterned PLA replicas on flat PLA plates and heating them in an oven at 80 °C for 20 min, thermally bonding the two parts without distorting the microchannel features. Tygon tubing (0.03 in. O.D.) was inserted into the access ports and sealed with epoxy glue to access the fluidic channels.

RESULTS AND DISCUSSION

Localization and patterning of the etching zone

Enzymatic micromachining can be controlled by establishing gradients in enzyme concentration that spatially regulate the etching process. This approach enables construction of microchannels whose height varies along the cross-section. This capability is enabled by the macromolecular architecture of PK that confines lateral diffusion ($D = 8.1 \times 10^{-7} \text{ cm}^2/\text{s}$ at 25 °C (Ref. 10)), enabling the dimensions of imprinted features to be maintained over a much greater downstream distance than possible using conventional small-molecule chemical etchants (e.g., $D = 1.6 \times 10^{-5} \text{ cm}^2/\text{s}$ for hydrofluoric acid (HF) etching of glass at 25 °C (Ref. 11)). Feature definition can be further controlled by co-injecting a stream containing an aqueous solution of BSA, establishing a competitive interaction that inactivates the laterally diffusing enzyme before it can attack the PLA (Fig. 1(a)). The resulting complexation between PK and BSA enables boundaries between the machined and protected regions to be sharply defined.

Patterning is therefore controlled by adjusting the relative concentrations and flow rates of enzyme and BSA in order to regulate the balance between convection and diffusion (expressed in terms of a Péclet number $Pe = uL/D$; where u , L , and D represent the characteristic flow velocity, diffusion length, and diffusion coefficient, respectively). In conventional laminar flow etching, convection-dominated confinement of small-molecule chemical etchants is only possible at high flow rates ($Pe \sim 5 \times 10^3$ for HF etching of glass at $u = 0.1$ m/s).^{12–14} In contrast, enzymatic machining employs macromolecular species that display greatly reduced diffusivities, enabling high Pe to be maintained at low flow rates ($Pe \sim 4 \times 10^3$, $u = 0.005$ m/s) to minimize reagent consumption and maintain pattern fidelity.

Physico-chemical model

To quantitatively capture the enzymatic micromachining process, we performed experiments using fluorescently labeled BSA to enable visualization of the lateral concentration profile (Fig. 1(b)). A concentrated 10% w/v solution, for example, experiences significant lateral transport into the adjacent stream. This effect acts to confine the etching zone by promoting strong complexation with PK. The underlying mechanics governing this interplay can be captured in terms of a convection-diffusion framework, yielding a system of differential equations expressing the concentrations of PK, BSA, and PK-BSA complexes (C_{PK} , C_{BSA} , and C_{PK-BSA} , respectively)

$$\frac{dC_{PK}}{dt} + \nabla \cdot (-D_{PK} \nabla C_{PK} + C_{PK} u) = k_{off} C_{PK-BSA} - k_{on} C_{PK} C_{BSA}, \quad (1)$$

$$\frac{dC_{BSA}}{dt} + \nabla \cdot (-D_{BSA} \nabla C_{BSA} + C_{BSA} u) = k_{off} C_{PK-BSA} - k_{on} C_{PK} C_{BSA}, \quad (2)$$

$$\frac{dC_{PK-BSA}}{dt} + \nabla \cdot (-D_{PK-BSA} \nabla C_{PK-BSA} + C_{PK-BSA} u) = k_{on} C_{PK} C_{BSA} - k_{off} C_{PK-BSA}. \quad (3)$$

Here, D_{PK} , D_{BSA} , and D_{PK-BSA} are the diffusivities of each species in solution, and the bulk fluid flow is directed axially along the length of the microchannel (the x -direction) with characteristic velocity u . The association and disassociation rate constants (k_{on} and k_{off} , respectively) represent PK complexation with BSA (i.e., $PK + BSA \xrightleftharpoons[k_{off}]{k_{on}} PK-BSA$).

The transport model in Equations (1)–(3) was numerically evaluated using COMSOL Multiphysics (version 4.0a; COMSOL, Inc.). Implementation of a fully 3D model incorporating a surface reaction boundary condition along the microchannel floor proved cumbersome and computationally intensive to implement. Consequently, we adopted a simplified 2D framework that captured the phenomena in a more efficient manner. First, the 2D steady-state velocity distribution over the microchannel cross-section ($50 \times 500 \mu\text{m}$ cross-section) was obtained. Next, Equations (1)–(3) were applied to obtain the 2D lateral species concentration profile in the x - y plane from inlet to outlet (i.e., looking down at the microchannel from above). This framework enabled the competitive interactions between PK-PLA and PK-BSA localized along the microchannel floor to be represented by a 2D model, subject to identifying a characteristic near-wall velocity u . The value of u was therefore treated as an adjustable parameter that captured the experimentally observed lateral FITC-BSA concentration profile at a distance of 1 cm downstream from the inlet (Fig. 1(b)), yielding a value of $u = 4.67 \times 10^{-4}$ m/s (corresponding to a distance of $1.1 \mu\text{m}$ from the PLA surface at a flow rate of $0.7 \mu\text{l/min}$, supplementary material Fig. S1¹⁹). This approach is further validated by noting that the simulated lateral distribution of [PK] concentration also closely matches experimentally obtained profiles of the etched PLA surface (Fig. 1(e)).

The laminar flow and viscoelastic material models were selected from COMSOL's model library to simulate geometry change of channel, and two-dimensional convection-diffusion mode was selected to numerically evaluate mass transport model. The computational domain was discretized into a mesh of quadrilateral elements. Properties of water at 37°C ($\rho = 993.36 \text{ kg/m}^3$, $\mu = 6.9 \times 10^{-4} \text{ Pa}\cdot\text{s}$) were used to represent all solutions (PK, buffer, BSA). Diffusion coefficients for PK, BSA, and PK-BSA complex were estimated to be 1.22×10^{-6} , 9.25×10^{-7} , and

$8.2 \times 10^{-7} \text{ cm}^2/\text{s}$, respectively, based on their molecular weight ($T=37^\circ\text{C}$).¹⁰ Association and disassociation rate constant values of $k_{on}=4 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{off}=10^{-4} \text{ s}^{-1}$ were assumed based on literature data.¹⁵

Despite its relative simplicity, this transport model yields results in quantitative agreement with experimental observations of the fluorescently labeled BSA concentration profile (Fig. 1(c)).

FIG. 1. Mechanism and physico-chemical model of enzymatic micromachining. (a) Microchannels with spatially varying cross-sectional profiles are constructed by co-injection of an enzyme (proteinase K, PK) and an inhibitor (bovine serum albumin, BSA) through a PDMS template bonded to a PLA substrate. The interface between co-injected PK and BSA streams establishes the boundary of the machined region. (b) Experimentally observed lateral concentration profile of FITC-BSA is obtained using fluorescence microscopy at three different BSA concentrations (images were acquired in a $500 \times 500 \mu\text{m}$ window located 1 cm downstream from the inlet, dashed line indicates centerline). (c) Predicted BSA concentration profile obtained by numerical simulation of the 2D convection-diffusion model in Equations (1)–(3) (dashed line indicates centerline). (d) Predicted concentration profile of free (uncomplexed) PK defining the effective etching zone (dashed line indicates etched boundary where the enzyme concentration approaches zero). (e) Profilometry measurements of machined profiles obtained 1 cm downstream from the inlet (black line; left ordinate) as a function of BSA concentration are overlaid with predicted lateral concentration profiles of free PK, PK-BSA complex (red and blue lines, respectively, inner right ordinate), and BSA normalized to the injected concentration (green line, outer right ordinate). Machining conditions: $[\text{PK}] = 6.92 \mu\text{M}$ in the bulk solution, $5 \mu\text{l}/\text{min}$, 37°C , 16 h, $[\text{BSA}]_0 = 0.15$ and 1.5 mM for 1% and 10% solutions, respectively.

Moreover, plotting the distribution of free (uncomplexed) proteinase K enables the extent of the etching zone to be predicted (Fig. 1(d)). This is evident in the corresponding experimentally obtained surface profiles, where the interfacial etching boundary corresponds to the location where the predicted proteinase K concentration drops toward zero (Fig. 1(e)). Based on these results, we found that a 1% w/v BSA concentration strikes an optimal balance between confinement of the etching zone and ability to maintain pattern fidelity over long downstream distances. We note that only hydrolyzed PLA remains after the etching process as the low-molecular-weight cleavage products become hydrolyzed and are released into the bulk along with dissociated PK,⁹ a mechanism supported by XPS spectra acquired from etched PLA surfaces (supplementary material Fig. S2¹⁹).

Machining of microchannels with irregular cross-section

Intricate topologies where feature depths vary along the microchannel cross-section can be fashioned by sequentially changing flow conditions and composition of the co-injected streams. We demonstrated this by employing a microchannel template with three independent inlets to fashion a cross-sectional profile incorporating a weir-like centerline barrier oriented parallel to the flow direction. Briefly, PK was infused into both of the outer streams, while BSA was introduced into center streams for 4.3 h at 37 °C (2.3 $\mu\text{m}/\text{h}$ degradation rate). This machining process yields the same depth (10 μm) on each side of the microchannel. When completed, this process produced a cross-sectional profile incorporating a center barrier along the entire length of microchannel with consistent pattern fidelity (Fig. 2(a)). Feature sizes can be further tuned by varying the relative flow rates of BSA and PK to hydrodynamically focus the desired streams (Fig. 2(b)). Enzymatic machining can also be applied to produce topologies incorporating continuously varying cross-section dimensions, challenging to build using conventional planar methods, by imposing suitable enzyme concentration gradients (Fig. 2(c)).

Uniform enzymatic machining of interior surfaces

The studies we have presented so far involve processes where etching is localized along the floor of a PLA substrate owing to the use of a PDMS microchannel template for flow of the PK etchant. Fully enclosed PLA networks can also be produced by first constructing a negative replica of the desired structure in PDMS, and then using this replica as a mold for subsequent fabrication in PLA (Fig. 3(a)). The imprinted microchannel is then thermally bonded to a flat PLA substrate to generate enclosed architectures (i.e., where all interior surfaces are composed of PLA). When PK is introduced into these systems, all surfaces are exposed to the etchant and experience machining, a unique feature of enzymatic micromachining in PLA.

We explored this process using planar microchannel networks in PLA. Since machining occurs along all interior surfaces exposed to flow of the enzymatic solution, etching can be characterized by the change in cross-sectional area between the initial ($w \times h$) and final ($w' \times h'$) states (Fig. 3(b)). These measurements were obtained from analysis of images obtained using a confocal microscope to observe the distribution of fluorescent dye inside the microchannel cross-section. All microchannels initially have a height of 28 μm , with widths of 42, 90, and 500 μm . We first performed a series of experiments involving straight channels to elucidate the effects of varying process parameters including etching time, temperature, and flow rate. Increasing the etching time results in an increase in cross-sectional area, achieving a degree of enlargement doubling the initial size occurring after 16 h (Fig. 3(c)). Higher temperatures accelerate the etching process, as compared with room temperature (Fig. 3(d)). Injecting the PK etchant at a higher flow rates also increase the channel surface area (Fig. 3(e)). We chose an enzyme concentration of 6.92 μM (0.2 mg/ml) based on previous characterization studies, where saturation of surface complexation sites was observed above this threshold.⁹ Based on these considerations, we selected 0.2 mg/ml of PK at 37 °C for 16 h at a flow rate of 0.2 $\mu\text{l}/\text{min}$ as optimal etching conditions for subsequent studies. We note that resolution in the depth dimension is defined by the etch rate (which can be reduced by performing the etching at reduced temperature), whereas lateral resolution is governed by the extent of hydrodynamic confinement achievable between co-

FIG. 2. A variety of cross-sectional topologies can be constructed. (a) Profilometry measurements as a function of downstream position confirm that etching profiles are maintained along the entire microchannel length. Scale bar = 1 mm. (b) Different flow rates can be applied in each stream to further control the size of the etched zone via hydrodynamic focusing (etching conditions same as in (a) except that flow rates of 10 and 0.5 $\mu\text{l}/\text{min}$ were applied in the PK and BSA streams, respectively; bar, 300 μm). (c) PK and buffer are co-injected into a microfluidic gradient-generating template and allowed to etch PLA at 37 $^{\circ}\text{C}$ for 16 h (22 $\mu\text{l}/\text{min}$, $Re = 0.16$, $Pe = 0.09$; bar in photo, 1 mm). The etched profile shows a continuously varying depth corresponding to the lateral concentration profile of PK (scale bar in SEM image, 300 μm).

injected enzyme and inhibitor streams. In previous work, we demonstrated confinement of enzymatic machining within a zone $\sim 4 \mu\text{m}$ -wide and 170 nm-deep using hydrodynamic focusing of PK between co-injected streams of BSA.⁹ Direct imaging via SEM also reveals that the initially rectangular microchannels etched channels assume more rounded profiles (Fig. 3(b)). The post-etch surfaces displayed a visually smooth appearance. Although we did not specifically quantify surface roughness here, we previously examined this issue and its dependence on substrate crystallinity,⁹ and we refer interested readers to this work for further details. The ability to produce

FIG. 3. Enzymatic machining of fully enclosed straight microchannels. (a) A negative PDMS mold is used to construct a microchannel replica in PLA, which is thermally bonded to a flat PLA substrate to produce an enclosed microchannel. (b) All interior surfaces are simultaneously etched upon injection of PK (above, schematic; below, SEM image, scale bar = $50 \mu\text{m}$). (c)–(e) Evolution of cross-sectional area under different etching conditions for microchannels with initial height $h = 28 \mu\text{m}$ and width $w = 42, 90, \text{ and } 500 \mu\text{m}$ (mean $\pm$ sd, $n = 3$). (c) Cross sectional area versus time ([PK] = $6.92 \mu\text{M}$ (0.2 mg/ml), $2 \mu\text{L/min}$, 37°C). (d) Cross sectional area versus temperature ([PK] = $6.92 \mu\text{M}$, $2 \mu\text{L/min}$, 16 h). (e) Cross sectional area versus flow rate ([PK] = $6.92 \mu\text{M}$, 37°C , 16 h).

microchannels with different cross-sectional topologies using a single design and mold introduces a dimension of flexibility not readily achievable using standard lithography, where different masks and molds are required to produce each individual design.

Enzymatic machining in microchannels incorporating branched networks

We further explored enzymatic micromachining in planar branched PLA microchannels to determine the extent to which etching rates can be locally varied within a complex network. A branched system was constructed consisting of 4 successive bifurcations, and cross-sectional area measurements were again used to quantify etching rates (Fig. 4(a)). Our measurements focused on the 90 and $42 \mu\text{m}$ -wide branches ($n = 2$ and 3 , respectively), as higher level branches became too small to accurately characterize within the resolution limits of our imaging approach. A key observation is that etching rates in these branches were consistently higher than in the straight channel controls (Fig. 4(b)). This may be attributable to the corresponding reduction in average velocity within each branch as the flow progressively splits between parallel channels, yielding an increased residence time for etching compared to the scenario in Fig. 3 where PK is injected into a single straight channel at the same flow rate. Another intriguing observation is that the topologies achieved in both the $n = 2$ and 3 branches after 16 h of etching are such that the hydraulic diameters appear to converge (Fig. 4(c), hydraulic diameter is defined as $4A/P$, where A is the cross-sectional area and P is the associated interior perimeter). In some cases, cross-sectional profiles evolve from the elliptical topologies observed in straight channels toward a pinched or “dog bone” shape (Fig. 4(d)), likely reflecting slower local flow velocities in the corner regions.¹⁶ These observations, while preliminary in nature, nevertheless suggest the capability to tailor the geometry of an initially templated network in order to achieve different internal microchannel dimensions upon completion of etching. Refinement of the computational model to include time-dependent erosion of the substrate interface offers one approach to validate and explain evolution of the etched cross-sectional topologies.

FIG. 4. Enzymatic machining of fully enclosed branched microchannels. (a) Planar branched microchannel incorporating 4 successive bifurcations ($n=1$: $w=500\ \mu\text{m}$; $n=2$: $w=90\ \mu\text{m}$; $n=3$: $w=42\ \mu\text{m}$; $n=4$: $w=24\ \mu\text{m}$). All of the channels have the same height of $28\ \mu\text{m}$. (b) Comparison between etching rates observed in the branched network and comparable straight channels of the same width at the same injection flow rate. (c) Comparison of hydraulic diameters observed in the branched network and comparable straight channels of the same width at the same injection flow rate. (d) SEM images depicting microchannel cross-sections in the branched network (dashed lines), revealing a “dog bone” shaped profile characterized by accelerated etching in the corner regions. Scale bars: before etching = $50\ \mu\text{m}$, after etching = $100\ \mu\text{m}$.

CONCLUSIONS

We have demonstrated the effectiveness of enzymatic micromachining using PK to tunably manipulate the cross-sectional dimensions of microchannels constructed in PLA, an inexpensive, rigid, and biodegradable substrate material (estimated fabrication costs are provided in the supplementary material¹⁹). We have also introduced a physico-chemical model that captures the flow-based machining process and enables etched profiles to be rationally predicted. The etching rate is influenced by parameters including temperature, local velocity, and enzyme concentration, suggesting a high degree of flexibility to produce locally tailored cross-sectional topologies via imposition of gradients and selection of the initial pre-etching geometry. These capabilities may be particularly useful in construction of bio-inspired 3D branched networks,^{17,18} enabling a variety of cell-favored interior environments to be produced using only a single template.

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