Electronic Supplementary Material (ESI) for Lab on a Chip. This journal is © The Royal Society of Chemistry 2023 Supplementary Table S1: Comparison of existing microbial lysis techniques and their efficacy.

Reference	Type of Lysis	Specifics	Demonstrated Microbial Applicability <sup>‡</sup>				
Number			Bacteria		Fungi	Pros	Cons
			Gram–	Gram+	i ungi		
3	Enzymatic	Zymolyase, 53°C 45 min	N/A	N/A	Y (S. cerevisiae)	Microfluidic Compatible	Premature Lysis (Low Temporal Control)
						Single-Cell Compatible	Species- Specific
4	Enzymatic	Zymolyase, 30°C 30 min	N/A	N/A	Y (S. cerevisiae)	Microfluidic Compatible Single-Cell Compatible	Species- Specific
5	Review (Mechanical, Thermal, Chemical, Enzymatic, Optical, Electrical)	Various Methods Described, Macro and Microscale	Y (E. coli)	Y (S. epidermidis, B. subtilis, M. smegmatis)	Y	See Table 3 and paper for an ov method's pros ar details macroscal Table 5 details r meth	I Table 5 of this erview of each nd cons. Table 3 le lysis methods, nicroscale lysis ods.
6	Mechanical	Nanoscale Barbs	Y (E. coli)	N/A	N/A	Microfluidic Compatible	Species- Specific
7	Mechanical	Nanoblade Arrays	N/A	N/A	N/A	Microfluidic Compatible	Not Applied to Microbes
8	Chemical, Mechanical	Porous Monolith, Detergent- Based Lysis Buffer	Y (E. coli)	Y (B. subtilis, E. faecalis)	N/A	Microfluidic Compatible	Species- Specific
9	Electrochemic al	Electrochemica I Hydroxide Generation	N/A	N/A	N/A	Microfluidic Compatible	Not Applied to Microbes
10	Chemical	Xylene, Acetone, Toluene Solution; Mixed via Magnetic Stirrer	Y (E. coli)	N/A	N/A	Microfluidic Compatible	Species- Specific
11	Mechanical	Bead Mill on a Compact Disk	Y (E. coli)	N/A	Y (S. cerevisiae)	Microfluidic Compatible	Difficult to fabricate, not in-line lysis
12	Mechanical	Bead Mill on a Compact Disk	Y (E. coli)	N/A	Y (S. cerevisiae)	Microfluidic Compatible	Difficult to fabricate, not in-line lysis
13	Review (Chemical, Mechanical, Electrical, Laser, Thermal, Acoustic, & Electrochemic al)	Various Methods Described	Y (E. coli)	Y (B. subtilis, B. anthracis, B. globigii,P. putida, S. epidermidis, S. mutans, M. tuberculosis)	N/A	See Table 1 of t overview of each and c	his paper for an n method's pros cons.
14	Review (Laser, Mechanical, Acoustic, Electrical, Chemical)	Various Methods Described	N/A	N/A	N/A	Microfluidic Compatible	Not Applied to Microbes
							Mechanical and acoustic methods leave

Chemical, &			large amounts
l nermal)			of cell debris.
			Chemical
			methods are
			often cell-type
			specific.
			Thermal
			methods are
			not viable for
			all cell types.

<sup>‡</sup>When available in the reference's primary text, the species that microbial applicability was demonstrated on has been included. "Y" is written as an abbreviation of "yes", to denote that the technique(s) mentioned in the reference has demonstrated microbial applicability, but the species the applicability was demonstrated on is not mentioned. N/A stands for not applicable, i.e., that microbial applicability of the technique was not demonstrated in the reference.



Supplementary FIG. S1. Summary of designs used for fabrication. (a) Four different layouts of KOH pyramids; (b) Pillars and Cryo pyramids; (c) Ridges; and (d) 3D printed mold to cast soft microfluidic device.



 $I \qquad Crushing efficiency \% = \frac{Number of Crushed Beads}{Number of Intact + Crushed Beads} \times 100$ 



Supplementary FIG. S2. SEM images show (a-b) beads being crushed or ripped off with broken silicon structures embedded into them, (c-d) beads, crushed or uncrushed, that are trapped between cylindrical pillars, (e-f) crushed beads trapped between ridges; fewer crushed beads are trapped when the ridges are perpendicular (f) to the direction of the flow compared to when with parallel with the direction of flow; (g-h) beads damaged by dense cryo pyramids, (i) nano-needles fabricated for 5 min etch, (j-k) relatively fragile nano-needles are generates when etched for 8 min. (I) Formula for calculating crushing efficiency from SEM images (top). The number of intact beads are counted first (bottom left); then the number of crushed beads is calculated from different-sized bead fragments (bottom right), binned and color-coded by size ( $\frac{1}{2}$  - blue,  $\frac{1}{4}$  - green,  $\frac{1}{6}$  - red, 1/16 - purple, and 1/32-white).

Supplementary Table S2: Primer sequences used for RT-qPCR of *C. albicans* and *S. cerevisiae*.

Species	Gene Symbol	Primer	Sequence (5'-3')
S. cerevisiae	ACT1	Forward	GATTCCGGTGATGGTGTTACTC
		Reverse	TCAAATCTCTACCGGCCAAAT
	UBC6	Forward	CCATACAAACCACCGGCTAT
		Reverse	CAGCCAGGATTCCAAGTATCA
	TDH3	Forward	GTCCACTCTTTGACTGCTACTC
		Reverse	GTGGAGGATGGGATGATGTTAC
C. albicans	ACT1	Forward	TTTCATCTTCTGTATCAGAGGAACTTATTT
		Reverse	ATGGGATGAATCATCAAACAAGAG
	LSC2	Forward	CGTCAACATCTTTGGTGGTATTGT
		Reverse	TTGGTGGCAGCAATTAAACCT
	ТПЦ1	Forward	CGGTCCATCCCACAAGGA
	ТОПТ	Reverse	AGTGGAAGATGGGATAATGTTACCA





Supplementary FIG. S3. Cq values of (a) S. cerevisiae and (b) C. albicans lysates and their dilutions used as positive controls (P.C.). In each case, 50 million cells were treated with zymolyase and sarkosyl for lysis. The lysates were purified and eluted in 100 µL buffer, and different dilutions of the lysate were amplified using ACT1, UBC6 and TDH3 primers for S. cerevisiae and ACT1, LSC2 and TDH3 primers for C. albicans that were designed for each microbial species.



ementary FIG. S4. Images of the ridges micropatterned silicon impactor chip when dry and with *S. cerevisiae* cells stuck on it after t = 5, 10, 15 min of piezo actuation.