Supplementary Note

BLAST mechanistic information

Cell membrane disruption using metallic nanoparticles under the illumination of short laser pulses has been widely studied[1-3]. On these metallic structures, the kinetic energy of oscillating electrons driven by electromagnetic waves quickly convert into lattice heat in picoseconds and starts to propagate into the surrounding aqueous media through thermal conduction[4,5]. Such rapid heating allows a substantial temperature rise in the nanostructure and the surrounding thin liquid layer over the laser pulse duration. Upon surpassing a threshold energy that superheats the liquid medium, part of the absorbed optical energy is converted into mechanical work through induction of explosive cavitation bubbles that generate highly localized and high-speed fluid flows. Depending on the pulse energy, pulse duration, and the metallic nanostructures, micron to nanoscale cavitation bubbles can be generated and shaped to trigger highly localized cell membrane disruption[1]. The degree of damage to cells induced by such a photothermal effect varies, depending on the size and the density of cavitation bubbles induced on a cell. Instant cell death occurs when there are too many bubbles induced or when the bubble size is too large. If the cavitation size and density is properly controlled, transient cell membrane permeability can be induced without killing the cells[2,3,6]. It should be noted is that if the laser pulse energy does not reach the threshold for triggering cavitation bubbles, the brief (tens of nanoseconds) transient temperature rise on nanostructures does not induce a significant effect on membrane permeability.

In BLAST, the crescent shaped titanium thin films coated on the sidewall of each SiO_2 hole aim to generate a cat-door-like cut to open a micron-sized membrane pore for large cargo delivery. The function of laser triggered cavitation bubbles is mainly to disrupt the cell membrane to create a pore for cargo delivery. The small volume perturbation (~ femtoliters) and random fluid flows induced by these bubbles play little role in introducing cargo into cells, especially for large-sized cargo.

The main challenge for cargo delivery using BLAST is to achieve uniform delivery across the entire cell population on the chip. Modern microfabrication technology can ensure that the dimensions of these micro and nanostructures on BLAST are highly uniform across a 1 cm^2 area, the current chip size. Laser pulsing needs to be done using a high repetition rate laser that scan the entire chip sufficiently rapidly such that the pores on the earlier scanned cells do not have time to reseal before allowing cargo delivery.

Uniform and active fluid delivery into cells across these transient pores is the most critical and challenging part in the design. There are hundreds of thousands of SiO_2 holes on a chip and tens of thousands of cells are randomly distributed. Holes fully covered or partially covered by cells have different flow resistances from holes not covered. How to ensure uniform delivery of cargo-carrying fluid into different cells is a major engineering challenge. The 3D microfluidic structures used in BLAST best address this challenge In the silicon structure, there are 10,000 vertical and short silicon channels [50 μm in diameter and 300 μm long (wafer thickness)] connecting the bottom cargo-storage reservoir and the SiO_2 membrane hole array. These wide, short, vertical channels are to ensure that once the bottom elastic cargo storage chamber is squeezed, the fluid pressure can quickly and even distribute across the entire chip such that all

SiO_2 holes are under the same pressure and cargo carrying fluid is pushed into the cell cytosol through the transient pores that are opened.

Fluid pumping needs to occur immediately after laser scanning is completed; otherwise, the membrane may reseal before cargo can be delivered into cells. The volume of fluid delivered needs to be experimentally optimized and calibrated. Large fluid deliveries cause immediate cell death and cell detachment. The fluid delivered along with desired cargo into a cell causes a small cell volume expansion. The elastic membrane then shrinks the cell volume back to its original size and shape. It is believed that some of the intracellular materials can also leak out through the transient membrane pores. On average, we estimate that 0.5 pl of fluid is delivered into cells, based on the number of beads delivered into a cell using a fluid with a known bead concentration (**Supplementary Fig. 4e**).

Another major advantage of BLAST is it is "clog-free" delivery for cargo with sizes smaller than the diameter of the SiO_2 membrane holes. We have never observed any clogging on the BLAST chip after numerous deliveries of all cargo types shown in this manuscript. This clog-free feature most likely can be attributed to the thin SiO_2 membrane, which is only 1.5 μm thick, shorter than the diameter of the hole. Hence there is no structure to cause clogging as the cargo passes through the holes.

References

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Supplementary Table 1 | Summary of all cell and cargo types delivered

Cell Type	Delivered Cargo	Efficiency	Viability
HeLa	Calcein	94.7±3.1 %	95.9±2.8 % †
	Dextran (40 kDa)	92.2±4.6 %	97.2±1.5 % ‡
	Gold particles (100 nm)	91.2±2.1 %	95.3±1.9 % †
	Magnetic beads (200 nm)	87.3±3.6 %	96.5±1.5 % †
	Francisella novicida	57.9±1.7 %	95.9±1.2 % ‡
NHDF	β-Lactamase (29 kDa)	96.1±2.5 %	97.5±0.9 % †
	Dextran (40 kDa)	96.6±2.5 %	97.7±2.2 % ‡
	Listeria	56.1±6.7 %	---
PB-MDM	Dextran (40 kDa)	56.0±7.3 %	94.6±1.5 % ‡
RPTEC	Dextran (40 kDa)	92.1±4.1 %	91.7±3.3 % ‡

†: Propidium iodide exclusion assay 6 h after BLAST delivery

‡: Propidium iodide exclusion assay 24 h after BLAST delivery

Supplementary Table 2 | Strains, plasmids, and primers used in this study

Strains, Plasmids, & Primers	Description	Source or Reference
Francisella tularensis* subspecies *novicida		
Fn	*F. novicida* Utah 112 strain	Ref. 7
Fn ΔFPI	Fn with the entire Francisella Pathogenicity Island deleted from the chromosome	Ref. 8
Fn Δ*iglC*	Fn with unmarked in-frame deletion of *iglC*	This study
sfGFP-Fn	Fn carrying pMP633-sfGFP	This study
sfGFP-Fn ΔFPI	Fn ΔFPI carrying pMP633-sfGFP	This study
sfGFP-Fn Δ*iglC*	Fn Δ*iglC* carrying pMP633-sfGFP	This study
iglC complemented sfGFP-Fn Δ*iglC*	Fn Δ*iglC* carrying pMP633BC-*iglC*-sfGFP	This study
Listeria monocytogenes		
DP-L2318	*Listeria monocytogenes* Δ*hly* Δ*plcB*; 10403S with in-frame deletion of genes encoding the listeriolysin O and the broad-range phospholipase C	Ref. 9
GFP-*L. monocytogenes*	DP-L2318 strain carrying pNF8	This study
Plasmids		
pMP590	Suicide vector for allelic replacement in *Francisella*; *sacB*; Kan^R	Ref. 10
pMP590-Δ*iglC*-ExC	pMP590 carrying an *iglC* gene deletion cassette containing the first 60 and the last 48 nucleotides of *iglC* and the ~1 kb chromosomal flanking regions	This study
pMP633	*Francisella* shuttle plasmid; Hyg^R	Ref. 10
pMP633-sfGFP	sfGFP driven by *bfr* (FTL_0617) promoter inserted in *Mlu*I site on pMP633	This study
pMP633BC-*iglC*-sfGFP	A bicistronic expression cassette of *iglC* and sfGFP driven by *bfr* (FTL_0617) and FTN_1451 promoter, respectively, inserted in the *Mlu*I site on pMP633	This study
pNF8	*Listeria* shuttle plasmid containing *gfp*-mut1; Em^R	Ref. 11
Primers	Nucleotide Sequence (5' – 3')	
Fn Δ*igl*C screening		
Δ*iglC*-ExC-F	AGTGAGATGATAACAAGACAACAG	
Δ*iglC*-ExC-R	TTACTATGCAGCTGCAATATATCCTA	
pMP590-Δ*iglC*-ExC construction		
Δ*iglC*-ExC-Up-F	TCGGGATCCATGGGTATGGTGGCAAAGAA	
Δ*iglC*-ExC-Up-R	TATCTGTGCTAGCAGTTCTCACATGAATGGTCT	

Δ*iglC*-ExC-Dn-F	GAGAACTGCTAGCACAGATAAAGGAGTTGCT
Δ*iglC*-ExC-Dn-R	GATCGCGGCCGCAAGCCGTAAAAACCGCACTA
pMP633-sfGFP construction	
Pbfr-Mlu-F1	ATACGCGTGGTACCTGGTTACTATTGCCATCATCACA
PbfrSD-SnaB-R1	CCTTTACTCATTACGTACCTCCTATTGTTACCTCCATTATTTAAAACTC
sfGFP-SnaB-F	AGGAGGTACGTAATGAGTAAAGGTGAAGAGCTATTTACTG
sfGFP-Mlu-SR	ATACGCGTGGATCCTCATTATTTATATAACTCATCCATTCCATGAGT
pMP633BC-*iglC*-sfGFP construction	
Pbfr-Mlu-F2	AGCTTACGCGTTGGTTACTATTGCCATCATCACAATAT
PbfrSD-SnaB-R2	CATTACGTACCTCCTATTGTTACCTCCATTATTTAAAACTC
iglC-SnaB-F	AGGAGGTACGTAATGAGTGAGATGATAACAAGACA
iglC-Age-SR	TACAACCGGTCTCGAGCAATTGCTATGCAGCTGCAATATATCCTA
Pomp-AgeF	TCGAGACCGGTTGTACATTAATTAATTTTGGGTTGTCACTCATCGTAT
Pomp-SacR	ACTCATTTTGAGCTCTCCTTTTTTTGTTATAAATATTTTAT
sfGFP-SacF	GAGAGCTCAAAATGAGTAAAGGTGAAGAGCTATTTACTG

Nucleotides that are underlined indicate restriction sites.

References

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