Supplementary Information

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Video S1. Human neutrophil swarming on an array of *E. coli* targets.

Video S2. Human neutrophil swarming toward an *E. coli* target with tracks showing neutrophil migration.

Video S3. Human neutrophils in the presence of snEVs.

Video S4. Human neutrophils in the presence of nEVs.

Video S5. Human neutrophils with no added EVs.

Table 1. Comparison of currently used techniques to analyze neutrophil migration.

Technique	Publication	Driving Force	Possible Quantification Methods
Boyden Chamber	Menezes, 2008.	fMLP gradient	Number of cells that passed through membrane
Under Agarose	Afonso, 2012.	fMLP gradient	Migration analysis through tracking individual cells
Microfluidic Devices, gradient driven	Movassagh, 2017.	fMLP gradient	Migration analysis through tracking individual cells
Microfluidic Devices, spontaneous	Ellett, 2018.	spontaneous migration	Migration analysis through tracking individual cells
Swarming Platform	Reategui, 2017.	neutrophil generated response to bioparticle	Migration analysis through tracking individual cells Collective behavior analysis (e.g., swarm size)

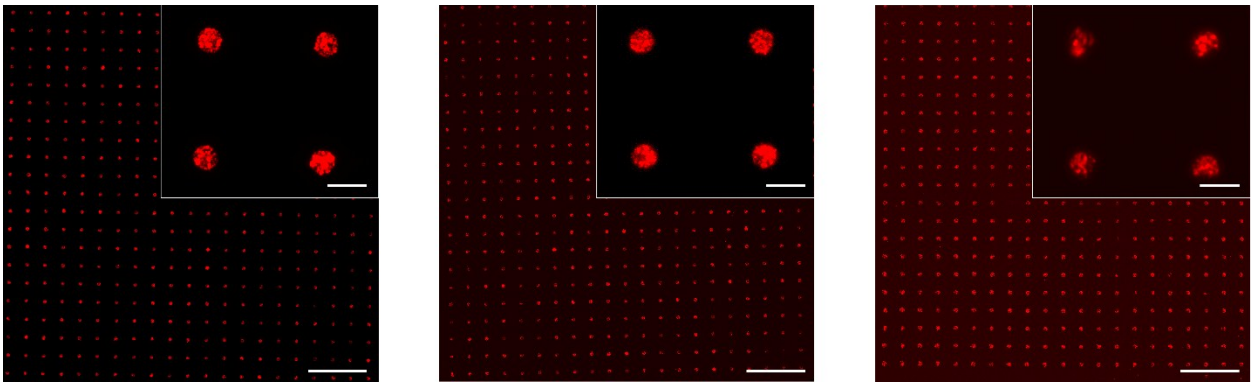

Fig. S1. Arrays of different microbial particles. Our platform can generate arrays of a variety of targets, including particles derived from *E. coli* **(a)**, *S. aureus* **(b)**, and *S. cerevisiae* **(c)**. (Scale bars: 500 μm main image, 50 μm inset).

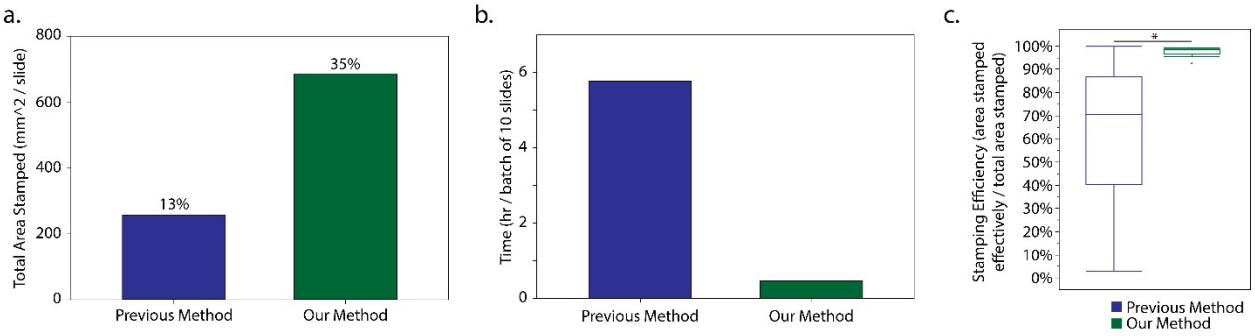

Fig. S2. Comparison of Different Bioparticle Array Generation Platforms. Our improved technology for generating bioparticle arrays is more efficient than the previously reported platform.[3] **a.** Our platform can produce a bioparticle array that covers 35% of the surface area of a glass slide while the previous platform covers 13%. (686 mm^2 stamped area/glass slide compared with 256 mm^2 stamped area/glass slide, respectively). **b.** The previous platform is more labor intensive than our platform. Our platform requires 8% of the labor of the previous platform (0.5 and 5.8 hours/batch of 10 slides, respectively). **c.** Our platform is more reliable and consistent across the array. On average, the previous platform has an efficiency of 62% ± 28%, while our platform has an efficiency of 98% ± 2%. This efficiency is calculated as the area stamped effectively divided by the total possible stamped area. (*t-test assuming unequal variances, $p < 0.00001$. Previous platform $n = 23$. Our platform $n = 11$)

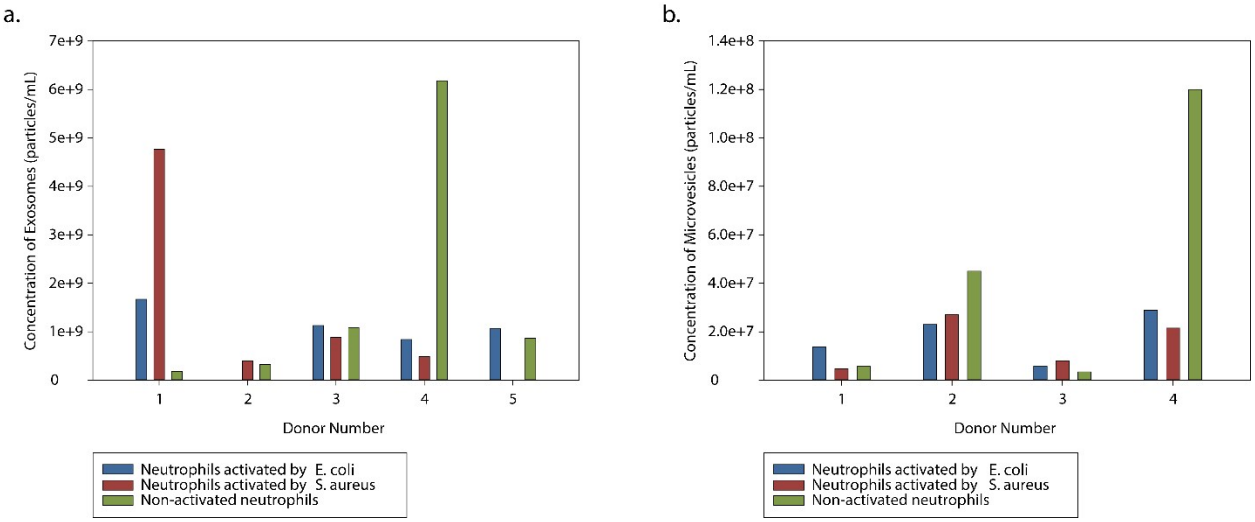

Fig. S3. Quantification of EVs Released by Neutrophils of Various Donors. a. The concentration of snEVs and nEVs in the exosome size range was determined for multiple donors when stable swarms had been produced (t = 90 min). There is no statistically significant difference between the concentration of snEVs and nEVs in the exosome size range at t = 90 min. (t-test, n = 5, $\alpha = 0.05$) **b.** The concentration of snEVs and nEVs in the microvesicle size range was determined for multiple donors when stable swarms had been produced (t = 90 min). There is no statistically significant difference between the concentration of snEVs and nEVs in the microvesicle size range at t = 90 min. (t-test, n = 4, $\alpha = 0.05$)

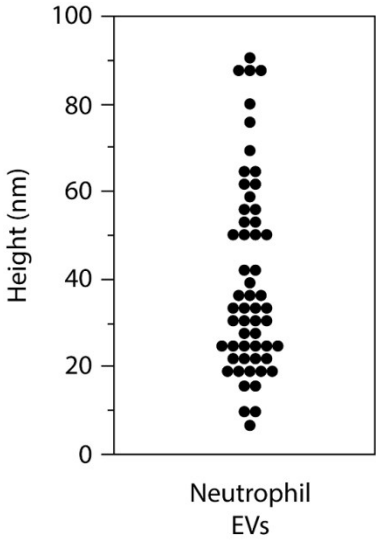

Fig. S4. Characterization of EV Heights by AFM. The height of the EVs on the AFM images range from 6 to 89 nm.

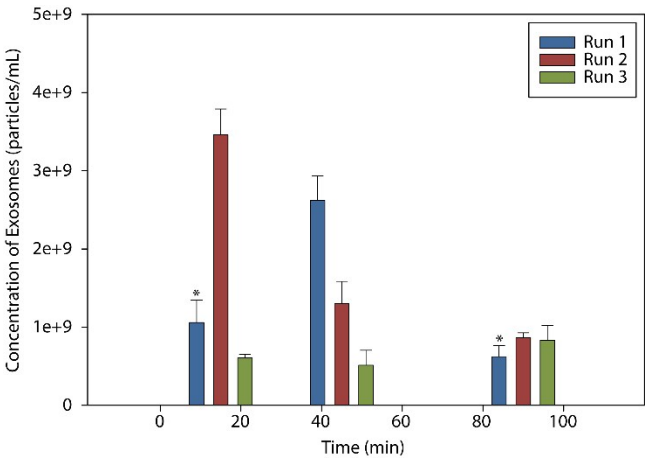

Fig. S5. Quantification of Exosomes Released Over Time by Non-Activated Neutrophils. The concentration of exosomes released by non-activated neutrophils followed no clear trend over time. (*one-sided t-test for the given time point compared to t = 45 for the same run, n = 3, $p < 0.05$).

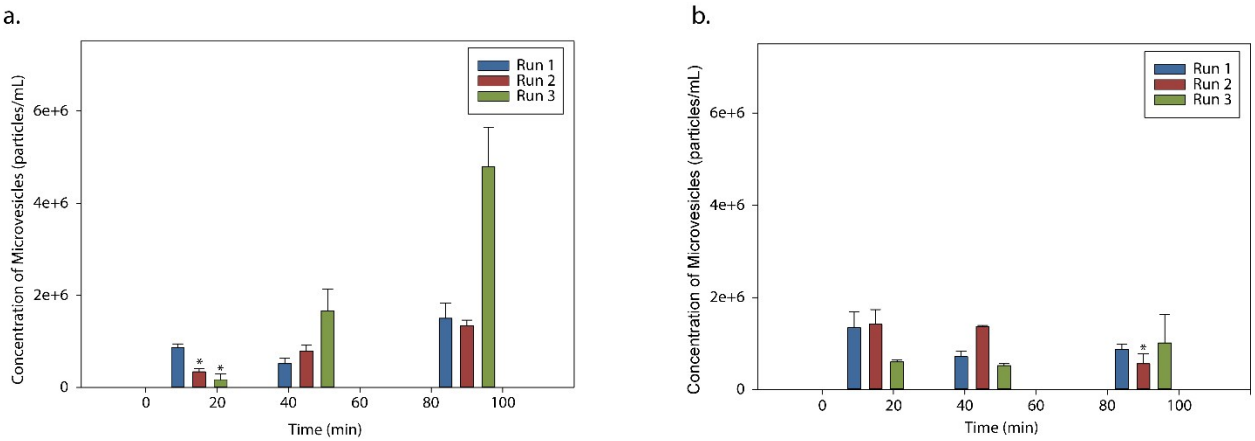

Fig. S6. Quantification of Microvesicles Released Over Time. The concentration of microvesicles followed no clear trend over time for both swarming (**a**) and non-activated (**b**) neutrophils. (*one-sided t-test for the given time point compared to t = 45 for the same run, n = 3, $p < 0.05$).

Video S1. Human neutrophil swarming on an array of *E. coli* targets. (Scale bar: 100 μm, original acquisition time: 30 min)

Video S2. Human neutrophil swarming toward an *E. coli* target with tracks showing neutrophil migration. (Scale bar: 30 μm, original acquisition time: 25 min)

Video S3. Human neutrophils in the presence of snEVs. (Scale bar: 50 μm, original acquisition time: 60 min)

Video S4. Human neutrophils in the presence of nEVs. (Scale bar: 50 μm, original acquisition time: 60 min)

Video S5. Human neutrophils with no added EVs. (Scale bar: 50 μm, original acquisition time: 60 min)

References

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