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Microbial separation from a complex matrix by a hand-held microfluidic device†

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Through a simple chemical activation of biomolecules present in the outer structures of microbial cells, microorganisms can be rapidly isolated on gold-coated surfaces in a microfluidic device with over 99% capture efficiency. Bacterial and fungal cells can be selectively captured, concentrated and retrieved for further analysis.

Despite important developments in rapid microbial detection technologies,[1–3] the implementation of these technologies at the industrial level remains very limited due to the complexity of real-world matrices (*e.g.*, food, agricultural or clinical specimens) and associated interferences that reduce selectivity and sensitivity of detection systems.[4] The development of upstream technologies capable of extracting and concentrating microorganisms from complex matrices prior to their identification and quantification is of paramount importance for the successful development of rapid diagnostics.

A number of methods have been developed for the separation and concentration of microorganisms. These methods include filtration followed by centrifugation[5,6] using cationic/anionic exchange resins, aqueous two-phase partitioning[7,8] and metal hydroxide-based separation.[9,10] While methods of physical separation have low specificity and are difficult to scale up, recently developed methods show promising capabilities (*e.g.*, immunomagnetic separation,[11,12] capillary electrophoresis,[13] acoustophoretic isolation,[14,15] ferromagnetic separation[16,17] and cell chromatography[18]). Despite the aforementioned developments, cell separation is still facing serious hurdles, such as increased cost and overall time of analysis, that hinder its use and commercialization. Here we show that simple, rapid reduction of the disulfide bonds, present in the microbial surface proteins, or deacetylation of chitin on the outer layers of fungi enables rapid cell capture on gold-coated surfaces (gold-coated slides). Following the capture, the cells can be analyzed directly on the slides or can be retrieved alive for further analysis by suitable detection assays.

The central facet to this novel cell separation system is the spontaneous bonding between gold and active moieties on the microbial surface, namely thiol and amine groups.[19,20] This bonding has been utilized in biosensing, nanoparticle synthesis and for the anchoring of biological molecules like antibodies and DNA to solid substrates.[21,22] Here we report the attachment of whole cells to gold-coated surfaces through a similar mechanism. Recent works in bioinformatics and genomic prediction[23] suggest the existence of disulfide bond-containing (Dsbc) proteins in the microbial outer layers in nearly all microorganisms except obligate anaerobes and intracellular parasites. In this study, Dsbc surface proteins of microorganisms were treated with a reducing agent, tris(2-carboxyethyl) phosphine (TCEP), to cleave the disulfide bond and yield free thiol groups. In a similar fashion, chitin, a surface biopolymer characteristic of fungi, was deacetylated with sodium hydroxide to convert chitin into chitosan, thus yielding free amine groups (Fig. 1). Additional information regarding procedures and experimental conditions applied can be found in ESI.†

To confirm the reaction between treated cells and gold-coated surfaces, various model organisms were studied, namely a Gram-positive bacterium (*Lactobacillus delbrueckii*), a Gram-negative bacterium (*Escherichia coli*), a non-filamentous yeast-fungus (*Saccharomyces boulardii*) and a filamentous mold-fungus (*Ceratocystis fagacearum*). For fabrication of gold-coated surfaces, standard microscope glass slides (25 mm × 75 mm) were coated on both sides with a layer of chromium (Cr, 5 nm-thick) followed by a layer of gold (Au, 50 nm-thick) using an E-Beam Evaporation System (SEC 600, CHA Industries, USA). Before use, the slides were cleaned with piranha solution, rinsed thoroughly with water and ethanol and dried under a stream of nitrogen. The slides were used immediately after cleaning. To reduce the bacterial Dsbc, *L. delbrueckii* and *E. coli* were treated with 2 mM TCEP and incubated for 10 min at room temperature. The treated cells were drop-casted onto gold-coated slides and left for 1 min to allow for

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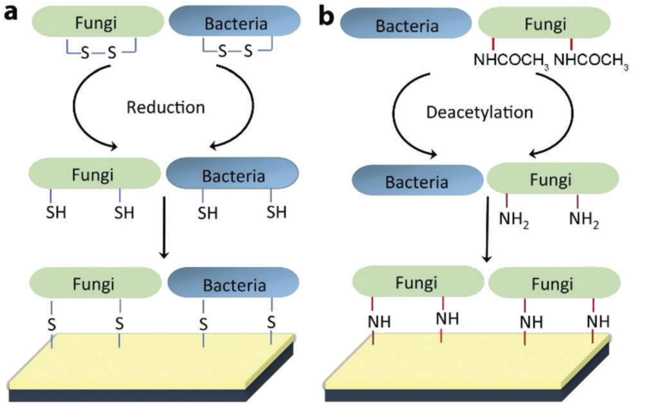

Fig. 1 Schematic illustration of the concept for microbial separation and concentration *via* reduction (a), and deacetylation of microbial surface biomolecules (b).

binding between the thiol groups and the gold surface. The slides were then rinsed thoroughly with water and analyzed by SEM (Fig. 2a). To confirm the ability to target fungi, we treated *S. boulardii* and *C. fagacearum* with NaOH. In particular, the cells were incubated in NaOH (4.8% or 9.6% w/w) for 10 min followed by neutralization of the alkali with hydrochloric acid to a pH around 8. Neutralization of NaOH served to provide the optimum conditions for the gold–amine interaction.[24] The suspensions of the deacetylated fungal cultures (10^7–10^8 CFU mL^{-1}) were deposited onto gold-coated slides for 1 min and were then rinsed thoroughly with water. The cells remained adhered to the surface due to the affinity of the amine groups to gold (Fig. 2a).

Based on our assumption, the presence of Dsbc surface proteins in both bacteria and fungi would also enable a separation of these microorganisms from a sample in a single reaction. To evaluate this aspect, we treated a mixture of the two bacterial and two fungal species with TCEP and deposited the obtained suspension (10^8 CFU mL^{-1}) onto the surface of a gold slide. After thoroughly rinsing the slides with water, both bacteria and fungi remained on the slide (Fig. 2b, left) and confirmed strong bonding between gold-coated slides and the free thiol groups present on both the bacteria and fungi.

A selectivity experiment was performed whereby a mixture of fungal and bacterial cultures (*S. boulardii*, *C. fagacearum*, *L. delbrueckii* and *E. coli*) was treated with NaOH for deacetylation of fungal chitin. As shown in Fig. 2b (right), only fungal cells remained adhered to the slides after rinsing with water. This finding makes the applied approach particularly promising for selective isolation and concentration of fungi.

Microbial separation and concentration methods usually aim at isolating the target microorganisms from the interfering matrix and concentrating them to detectable levels. Such methods enable analysis of diluted or large volume samples within a reasonable time and provide the possibility to use the pre-concentrated sample for analysis by compatible quantitative sensing platforms. It is worth noting that immunoassays and molecular detection methods (*e.g.*, polymerase chain reaction and enzyme linked immunosorbent assay) are limited in sample volume (one hundred microliters or less), imposing specific requirements for the degree of sample pre-concentration. The concept demonstrated above shows potential to isolate microbial agents from sample matrices without the need for culture enrichment. After validating the concept of microbial capture on gold surfaces, a microfluidic device was designed to solve the challenge of processing large volumes. The device was inspired from a plate heat exchanger whereby fluid flows through a narrow channel between two plates. Similarly, we fabricated a device comprised of an integral flow microchannel that followed a switchback pattern with a series of sharp turns. The top and bottom of this microchannel was confined within two gold-coated slides which served as the reactive surfaces (Fig. 3). The microchannel-based design of the device aimed at providing maximum contact surface between the slides and the treated cell suspensions. The device chamber was designed using an open source 3D modelling software and printed with a stereolithography (SLA) based 3D printer (Form 2, Formlabs). The major parameter that was optimized during this study was the width (inter-slide distance) of the microchannel within the device chamber. Due to material constraints and printer limitations, the smallest channel width used was 250 μm. From that starting width, devices were fabricated with microchannel widths of 350, 500, 600, and 700 μm. Each device was optimized to contain six slides, thus allowing for enough space within the

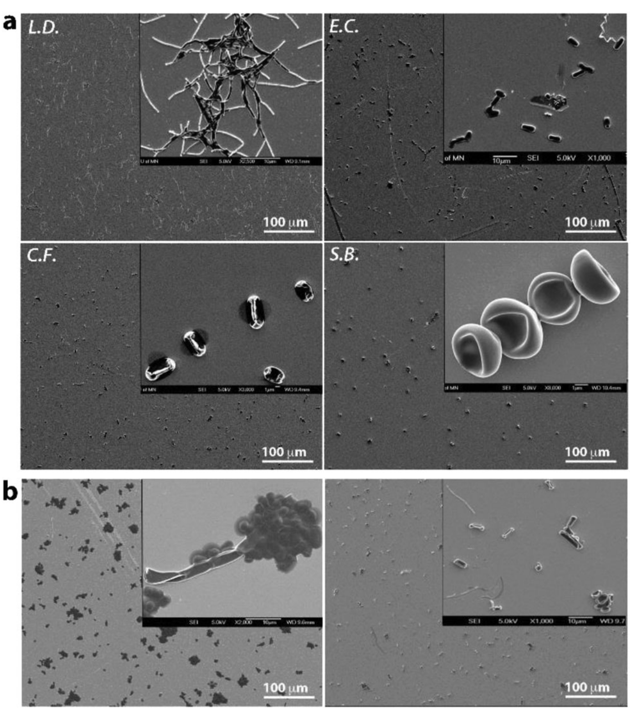

Fig. 2 SEM images of microbial capture on Au-coated glass slides. (a) SEM images showing the capture of a Gram-positive bacterium *L. delbrueckii* (L.D.), a Gram-negative bacterium *E. coli* (E.C.), a filamentous fungus-mold *C. fagacearum* (C.F.), and a non-filamentous fungus-yeast *S. boulardii* (S.B.) on Au-coated glass slides. (b) SEM images of captured bacterial and fungal cells/spores from a mixture of *L. delbrueckii*, *E. coli*, *S. boulardii and C. fagacearum* after reduction with TCEP (left image), and the selective capture of fungal hyphae/spores (*S. boulardii* and *C. fagacearum*) after deacetylation of the microbial mixture with NaOH (right image).

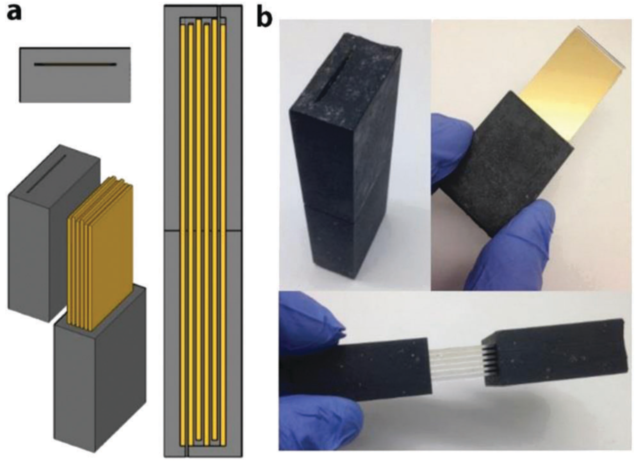

Fig. 3 Design and printed prototype of the hand-held device for the separation and concentration of microorganisms. (a) The schematic shows the view of the device, horizontal cross-sections showing the gold-coated glass slides positioned inside the chamber with an integral channel. (b) Photograph of the 3D-printed prototype. The device can contain several gold-coated slides and a sample volume ranging from 1 mL to 10 mL.

device to contain at least 1 mL of a liquid sample (1 cm^3 free space within the device chamber) at its narrowest channel width (250 μm). Devices with wider channels allowed for the treatment of up to 10 mL of sample volumes (Fig. 3). Microchannel width and treatment time were optimized using *Lactobacillus delbrueckii* as a model microorganism (Fig. 4a). The capture efficiency of the device was studied for aqueous suspensions of the bacterial cells (prepared by the reduction process) incubated in the device chamber for time periods ranging from 10 s to 60 min. The experimental values of capture efficiency in percentage were determined by measuring the optical density (OD_{600}) of the suspensions before and after exposure to the gold slidpes. Three major observations were made from Fig. 4a: (i) The capture efficiency of the device ranged from 23 to 44% when the cells were immediately passed (contact time 10 s) through the microchannels, revealing spontaneous attachment of cells to the adjacent microchannel walls (slides). (ii) After 30 min of incubation, all device designs exhibited the same capture efficiency (75–80%) regardless of the microchannel width. The correlation trend between the microchannel width and the device capture efficiency seemed to be reverse after 30 min of incubation. (iii) After 60 min of incubation, capture efficiency had direct dependency on the microchannel width, reaching the highest value (99.3%) for 600 μm-microchannel device (Fig. 4b). The slight decrease in the capture efficiency to ~91%, observed for the 700 μm-microchannel device, we attributed to microbial saturation on the slides. Control experiments with non-reduced microbial cells were performed using the 600 μm-microchannel device and *S. boulardii* incubated in the device chamber for 1 h. Capture efficiencies for the reduced and non-reduced *S. boulardii* were 87.1% and 33.3%, respectively (Table S2, ESI†). This finding yielded several essential conclusions: (i) the loading rate of the gold surface with the cells that have chemically treated disulfide

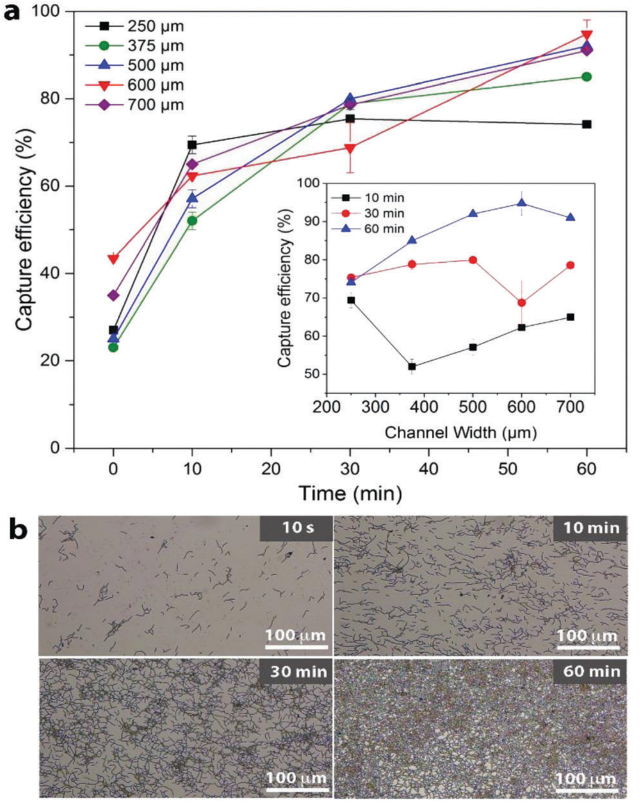

Fig. 4 Microbial capture with the microfluidic device. (a) Effect of the incubation time and microchannel width on microbial capture efficiency within the device. (b) Optical images of *L. delbrueckii* cells captured on the Au-coated slides of the microfluidic device at different incubation times.

bonds on their surface is almost three-fold greater than loading with cells that have untreated disulfide bonds. (ii) The surface proteins of microbial cells have a significant amount of inherent exposed functional moieties that have high affinity to gold (*e.g.*, amine groups). (iii) The capture efficiency of the device varies for the different microorganisms and suggests that each species has a unique amount and/or distribution of Dsbc proteins in its outer layers. At this time, more experimental work and fluid dynamic modelling is needed to understand the impact of diffusion and microbial motility on these variations.

Once the microorganisms were captured on the Au-coated glass slides, they can be used directly for microscopic identification or quantification on the slides, or can be retrieved into solution for analysis with other methods. Cell retrieval and viability studies were conducted with *S. boulardii* and *E. coli* cells after the capture experiments. To elute the captured cells from slides of the device, the slides were soaked in 100 mM NaCl for 10 min. Sodium chloride was used here as it has been already reported by Bhatt[25] that 100 mM NaCl favors the dissociation of the thiol–Au bond. After centrifugation of the obtained eluates to remove NaCl, the resulting solutions were inoculated into growth media (YM broth and TSB). The cultured cells were found to be viable as their growth was observed in 16–24 h with the rate similar to the growth of the untreated cells (ESI,† Tables S1, S3 and S4).

To demonstrate the microfluidic system for microbial separation and concentration on real-world samples, microbial separation was performed on yogurt (''Yoplait Original'' yogurt, Yoplait USA, Inc.) and wood shavings from an infected oak tree (the sample bearing the fungus *C. fagacearum*). Yogurt was used as a sample matrix which naturally contains bacterial cultures that could interfere with fungal capture in the device. A yogurt sample was diluted in water at a 1 : 3 ratio to allow for easy flow through the device; then it was treated with TCEP and passed through the device with an incubation time of 10 min. Optical images of a gold slide with captured bacteria directly from the yogurt sample are shown in Fig. S1a (ESI†). In contrast, when the yogurt sample was deacetylated and passed through the device, no cell capture was observed on the slides (Fig. S1b, ESI†). However, when the yogurt sample was artificially inoculated with the yeast culture (*S. boulardii*) and mold (*C. fagacearum*) followed by the deacetylation procedure, captured *S. boulardii* spores (Fig. S1c, ESI†) and *C. fagacearum* hyphae and spores (Fig. S1d, ESI†) were found on the slides. In a similar manner, deacetylation was successfully done to treat and capture *C. fagacearum* from wood shavings. Like the yogurt sample, both spores and hyphae were observed on the slides (Fig. S1e, ESI†).

In summary, a novel microbial separation concept and a hand-held microfluidic device for the separation and concentration of microorganisms from model solutions and complex matrices were introduced. The concept exploits the existence of disulfide bond-containing surface proteins on the outer layers of microorganisms. A simple treatment of the microbial sample with a reducing agent leads to spontaneous attachment of the microorganisms to a gold-coated surface. The proposed concept and device enables the capture of up to 99.3% of the microbial cells from the studied samples (*L. delbrueckii*) onto Au-coated glass slides within 60 min. Fungal cells were captured by the microfluidic device using a deacetylation method for converting fungal chitin into chitosan. The proposed method could be applied for deferential concentration of fungal species from samples containing diverse microbial populations. This novel method can also be used for immobilization of whole and viable cells for further bioprocessing. Furthermore, the method could help advance the rapid detection of various microbial contamination such as fungal diseases in plants and trees, the detection of fungi in dairy products or the distinction between fungal and bacterial sepsis in clinical settings.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

1 F. Yeni, S. Acar, Ö. G. Polat, Y. Soyer and H. Alpas, *Food Control*, 2014, **40**, 359–367.
2 V. Velusamy, K. Arshak, O. Korostynska, K. Oliwa and C. Adley, *Biotechnol. Adv.*, 2010, **28**, 232–254.
3 V. Koubová, E. Brynda, L. Karasová, J. Škvor, J. Homola, J. Dostálek, P. Tobiška and J. Rošický, *Sens. Actuators, B*, 2001, **74**, 100–105.
4 B. Brehm-Stecher, C. Young, L. A. Jaykus and M. L. Tortorello, *J. Food Prot.*, 2009, **72**, 1774–1789.
5 K. Venkateswaran, Y. Kamijoh, E. Ohashi and H. Nakanishi, *Appl. Environ. Microbiol.*, 1997, **63**, 4127–4131.
6 N. Rijpens, L. Herman, F. Vereecken, G. Jannes, J. De Smedt and L. De Zutter, *Int. J. Food Microbiol.*, 1999, **46**, 37–44.
7 R. Hatti-Kaul, *Mol. Biotechnol.*, 2001, **19**, 269–277.
8 P.-G. Lantz, F. Tjerneld, E. Borch, B. Hahn-Hägerdal and P. Rådström, *Appl. Environ. Microbiol.*, 1994, **60**, 3416–3418.
9 L. A. Lucore, M. A. Cullison and L.-A. Jaykus, *Appl. Environ. Microbiol.*, 2000, **66**, 1769–1776.
10 M. A. Cullison and L.-A. Jaykus, *J. Food Prot.*, 2002, **65**, 1806–1810.
11 C. Logue, J. Sherwood, P. Olah, L. Elijah and M. Dockter, *J. Appl. Microbiol.*, 2003, **94**, 16–24.
12 R. Reinders, A. Barna, L. Lipman and P. Bijker, *J. Appl. Microbiol.*, 2002, **92**, 1015–1020.
13 M. J. Desai and D. W. Armstrong, *Microbiol. Mol. Biol. Rev.*, 2003, **67**, 38–51.
14 B. Ngamsom, M. J. Lopez-Martinez, J.-C. Raymond, P. Broyer, P. Patel and N. Pamme, *J. Microbiol. Methods*, 2016, **123**, 79–86.
15 M. A. Md Ali, K. Ostrikov, F. A. Khalid, B. Y. Majlis and A. A. Kayani, *RSC Adv.*, 2016, **6**, 113066–113094.
16 Z. M. Wang, R. G. Wu, Z. P. Wang and R. V. Ramanujan, *Sci. Rep.*, 2016, **6**, 26945.
17 A. R. Kose and H. Koser, *Lab Chip*, 2012, **12**, 190–196.
18 M. B. Dainiak, F. M. Plieva, I. Y. Galaev, R. Hatti-Kaul and B. Mattiasson, *Biotechnol. Prog.*, 2005, **21**, 644–649.
19 F. Chen, X. Li, J. Hihath, Z. Huang and N. Tao, *J. Am. Chem. Soc.*, 2006, **128**, 15874–15881.
20 S. L. Westcott, S. J. Oldenburg, T. R. Lee and N. J. Halas, *Langmuir*, 1998, **14**, 5396–5401.
21 M.-P. N. Bui, S. Ahmed and A. Abbas, *Nano Lett.*, 2015, **15**, 6239–6246.
22 Z. Li, R. Jin, C. A. Mirkin and R. L. Letsinger, *Nucleic Acids Res.*, 2002, **30**, 1558–1562.
23 R. J. Dutton, D. Boyd, M. Berkmen and J. Beckwith, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 11933–11938.
24 D. P. Nair, M. Podgórski, S. Chatani, T. Gong, W. Xi, C. R. Fenoli and C. N. Bowman, *Chem. Mater.*, 2014, **26**, 724–744.
25 N. Bhatt, P.-J. J. Huang, N. Dave and J. Liu, *Langmuir*, 2011, **27**, 6132–6137.

