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Cancer bioimprinting and cell shape recognition for diagnosis and targeted treatment

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Jevan Medlock,[a] Anupam A. K. Das,[a] Leigh A. Madden,[b] David J. Allsup[c] and Vesselin N. Paunov*[,a]

Cancer incidence and mortality have both increased in the last decade and are predicted to continue to rise. Diagnosis and treatment of cancers are often hampered by the inability to specifically target neoplastic cells. Bioimprinting is a promising new approach to overcome shortfalls in cancer targeting. Highly specific recognition cavities can be made into polymer matrices to mimic lock-and-key actions seen in *in-vivo* biological systems. Early studies concentrated on molecules and were inhibited by template size complexity. Surface imprinting allows the capture of increasingly complex motifs from polypeptides to single cell organisms and mammalian cells. Highly specific cell shape recognition can also be achieved by cell interaction with imprints that can be made into polymer matrices to mimic biological systems at a molecular level. Bioimprinting has also been used to achieve nanometre scale resolution imaging of cancer cells. Studies of bioimprint-based drug delivery on cancer cells have been recently trialled *in-vitro* and show that this approach can potentially improve existing chemotherapeutic approaches. This review focuses on the possible applications of bioimprinting with particular regards to cancer understanding, diagnosis and therapy. Cell imprints, incorporated into biosensors can allow to improve the limits of detection or negate the need for extensive patient sample processing. Similar cell imprinting platforms can be used for nanoscale imaging of cancer morphology, as well as to investigate topographical signalling of cancer cells *in-vitro*. Lastly, bioimprints also have applications as selective drug delivery vehicles to tumours with the potential to decrease chemotherapy-related side effects.

Introduction

1. Cancer and incidence

Cancer is the collective term for a range of conditions characterised by a deregulated proliferation of defective cells caused by somatic genomic mutations[1–3] resulting in compromised mitotic cell division. Worldwide, approximately 14.1 million people were diagnosed with cancer in 2012.[4–6] This figure had increased from 12.7 million in 2008 and is estimated to increase to around 22 million by 2030 largely due increased life expectancy.[6,7] Cancer is the second highest cause of premature death in the U.K.[6] Cancer treatment relies on a combination of surgery, radio- and chemotherapy, which are variably effective and often associated with significant morbidity.[1] All are invasive and, particularly chemotherapy, causes severe discomfort to patients. In 2014 more than 50% of cancer cases reported in the U.K. were in patients over 70 making them unsuitable for the preferred intense treatments.[6,8] Improved diagnostics and therapy mean cancer survival has more than doubled in the last 40 years.[6] However, outcomes vary dramatically between different patients. On average, as few as 1% of pancreatic cancer patients are expected to survive 10 years after their diagnosis date, mainly due to the late presentation of symptoms.[6]

1.1 Diagnostics and cell targeting

Current cancer diagnosis relies on a variety of imaging techniques, such as X-rays, magnetic resonance imaging (MRI), computerised tomography (CT) and positron emission tomography (PET)[9], which provide non-invasive methods to identify neoplasms in patients. Other, more invasive techniques, involve removal and analysis of cancerous tissue by biopsy or assessment of peripheral blood.[1–3,9,10] Recent studies have investigated circulating tumour cells (CTCs) that have become detached from the primary tumour[11–14] and spread through the body in the blood during metastasis.[13] These cells from solid tumours differ from liquid tumours such as leukaemia where the primary tumour is of fluid tissue.[15] CTCs have been identified as a possible surrogate biomarker indicative of the primary cancer site, spread of the disease and patient prognosis.[11,13] Identification, characterisation and enumeration of CTCs from peripheral blood samples yields an opportunity for better understanding of cancer biology and the metastatic process. This makes CTCs a possible marker for early cancer detection and prognosis. For non-blood cancers, typically 1 in 10^9 peripheral blood cells are cancerous.[16,17]

[a] School of Mathematics and Physical Sciences (Chemistry), University of Hull, Cottingham Road, Hull, HU67RX, UK;
[b] School of Life Sciences (Biology), University of Hull, Cottingham Road, Hull, HU67RX, UK.
[c] Hull York Medical School, University of Hull, Cottingham Road, HU67RX, Hull, UK.
*Correspondence author: V.N.Paunov@hull.ac.uk, Fax: +44 (0)1482 466410; Tel: +44 (0)1482 465660.

Mr Jevan Medlock obtained a MChem degree in Chemistry from the University of Hull in 2014. Currently, he is a PhD student under the supervision of Professor Vesselin Paunov. His PhD studies involve bioimprinting of blood cancer cells and characterisation of the interactions between the cancer cells and their imprints. His research is focussed on therapy of acute myeloid leukaemia.

Dr Anupam Das received a PhD in Physical Chemistry in 2014 from the University of Hull under the supervision of Professor Vesselin Paunov. He did two years postdoc at the Department of Chemistry of the University of Hull on interdisciplinary projects of industrial interest. His research interests include biomaterials, functionalisation of cells with nanoparticles and polymers, preparation of living materials from symbiotic cells. He is currently a CRUK-funded postdoctoral researcher at the School of Mathematics and Physical Sciences at the University of Hull. His current project involves using bioimprinting technology for removal of myeloblasts from blood of acute myeloid leukaemia patients.

Dr Leigh A Madden received a PhD from the University of Hull, UK in 1997. He then continued this work in biodegradable polymers for a further 2 years in a position funded by Monsanto Inc. After this he moved into the medical field at the Postgraduate Medical Institute at the University of Hull conducting research into a range of diseases but with a primary focus on cancer. Now working in the new School of Life Sciences at the University of Hull, his current research interests include the investigation of cellular microvesicles in cancer related coagulation and also the application of chemistry derived technology such as microfluidics and bioimprinting to medical research.

Dr David Allsup undertook his PhD on "B-cell receptor signalling in CLL" from the University of Liverpool. He worked 2000-2004 as a clinical lecturer at the University of Liverpool. Since 2004 he has been a Consultant Haematologist in Hull and East Yorkshire Hospitals NHS Trust. His research interests include malignancies of the hematologic and immunologic systems. His present clinical research is focussed on signalling pathways and susceptibility loci in chronic lymphocytic leukaemia. He has authored more than 40 scientific papers and book chapters.

Prof Vesselin N. Paunov received his PhD in Physical Chemistry in 1997 from the University of Sofia, Bulgaria. He worked as a visiting researcher at the Universities of Patras, Greece, and Erlangen, Germany and as a postdoctoral researcher at the University of Delaware. In 2000 he took an academic post at the University of Hull, UK) where he is currently a Professor of Physical Chemistry and Advanced Materials at the School of Mathematics and Physical Sciences (Chemistry). His present research interests include formulation science, microencapsulation, triggered release of actives, antimicrobial nanoparticles, colloid antibodies for cell shape recognition and selective targeting. His ongoing research is focused on synthetic colloid particles with antimicrobial action, whole cell bioimprinting techniques and bioimprint-cell interactions. At present, he has published over 130 research articles that have received more than 6000 citations.

1.2 Purpose of the review

Cancer incidence and mortality are increasing worldwide despite improved diagnosis and therapy. The main disadvantage of many conventional chemotherapeutic approaches is a lack of selectivity towards the malignant cell, with considerable resultant toxicity as a result of damage to normal, non-cancerous tissues. This review will evaluate techniques currently used by clinicians to target cancer and show the recent advances made in bioimprinting technology, which may prove an alternative modality for selectivity in cell separation, diagnosis and targeted treatment of cancer.

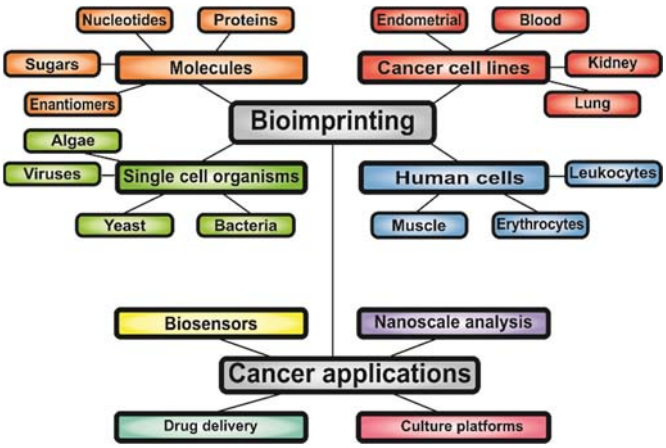

Fig. 1 Schematic of the development of bioimprinting technology from relatively rudimentary templates through imprinting to whole cells and possible applications in cancer diagnostics and therapy.

The current types and applications of bioimprinting are presented in Fig. 1. These range from imprinting of molecules on surfaces to whole cell imprinting and their applications in improving cancer diagnostics and therapy.

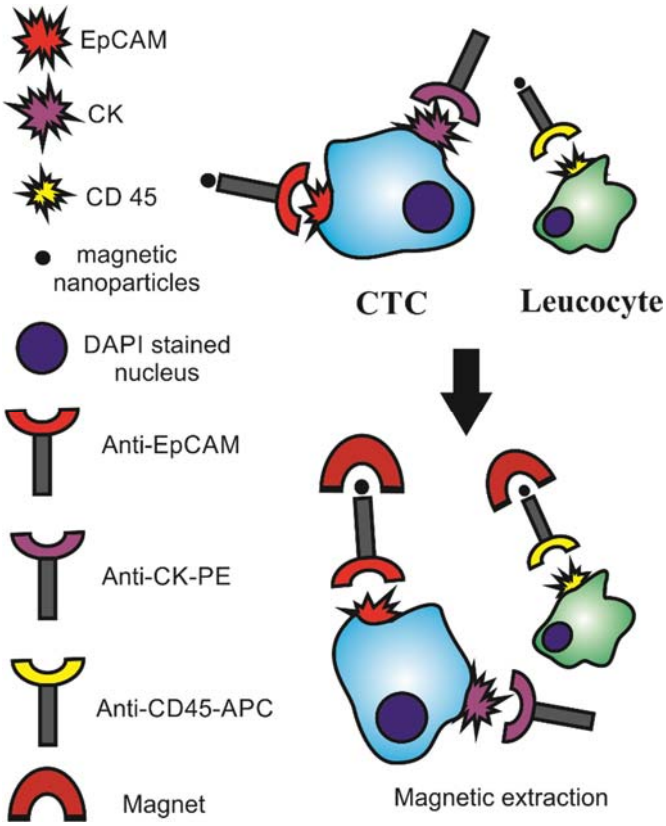

Fig. 2 Schematic showing the CellSearch technology for the extraction of CTCs from human blood. CTC, circulating tumour cell: EpCAM, epithelial cell adhesion molecule; CK-PE, cytokeratins–phytoerythrin; CD45-APC, cluster of differentiation 45-allophycocyanin; DAPI, nucleus stained with DAPI.[15] Redrawn with permission from Ref. 15.

2. Current methods of cell sorting and targeting

Current methods of cell separation exploit a combination of chemical and physical differences between the cell types. Physical sorting methods typically require large differences in properties, like cell size, shape or density to enable the deployment of methodologies based on either microfiltration or density centrifugation.[16,18] However, these tend not to be highly selective for specific cell-types. For instance, Ficoll-Paque™ extraction of PBMCs (peripheral blood mononuclear cells) from whole blood can separate erythrocytes, plasma and leukocytes, but does not discriminate between mononuclear cells such as lymphocytes and monocytes.[18,19] When used in sample preparation, large sample volumes are needed or subsequent cell culturing to expand cell numbers, which complicates analysis, making it more time consuming and cost intensive. More specific methods of cell separation can be employed by utilising monoclonal antibodies (Mabs) engineered to specifically target cancer-associated antigens. However, Mabs can be very expensive to generate and often have little activity as monotherapy, with the best results being obtained by combining Mabs with conventional chemotherapy to form chemo-immunotherapy, often with significant associated chemotherapy-induced toxicity.[85]

2.1 Label free cell isolation

Various microfluidic devices have been reported for isolation of rare cells on account of hydrodynamic properties in a field known as label-free isolation.[20] For instance, Chung *et al.* created microfluidic devices incorporated with powerful magnetic filters and size based cell sorters.[21] The microfluidic design of the device ensured efficient and accurate capture of cancerous cells from whole blood.[21] Chen *et al.* also used a microfluidic device and exploited the differences in the hydrodynamic forces acting on cells of different size. Deterministic lateral displacement arrays allowed rapid isolation of cancer cells from diluted whole blood samples.[17] Circulating liver cancer cells were trapped by size gradated microfluidic channels termed a 'mechanical sieve' by Moon *et al*.[22] The study characterised cancer progression by enumerating CTCs per millilitre of whole blood. The method showed an excellent precision, with a single cancerous cell per millilitre of blood detected and a 98.9% recovery of cancer cells. Isolation used small sample volumes (typically 4 ml) easily obtainable in clinical practice. Such classification can be a key prognostic marker in cancer treatment.[22]

Chemical identification and separation use unique cell membrane features. Cell separation using a microfluidic device based technology was found to be superior compared to the macroscale technologies due to higher cell-substrate interaction[18,23] and flow techniques[23] with off-chip purification in order to obtain target cancer cells with enhanced purity.[24] Isolation of CTCs can lead to an improved understanding of cancer progression, communication and therapy.

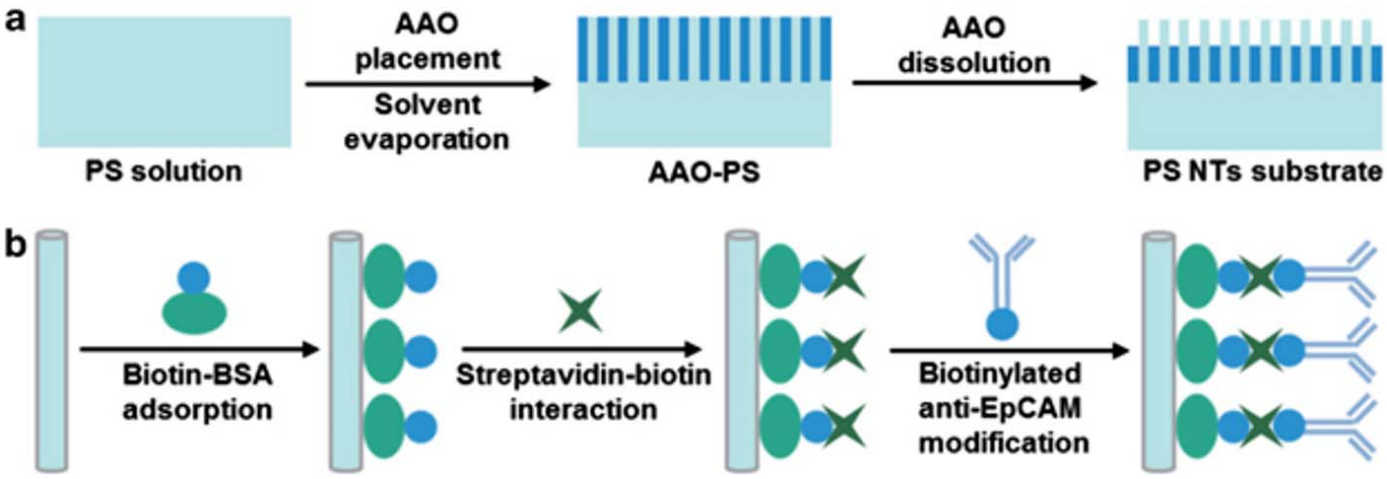

Fig. 3 Schematics showing the production and modification of soft polystyrene (PS) nanotube (NT) substrate. (a) Fabrication process of the PS NT substrate using the replication method with anodic aluminium oxide (AAO) as the template and (b) functionalisation of the PS NT substrate surface with anti-epithelial cell adhesion molecule (EpCAM).[31] Reproduced from reference [31] with permission from [Nature Publishing Group], copyright [2013].

Currently, the only the U.S. Food and Drug Administration (FDA) approved CTC extraction system that is commercially available, is called CellSearch™.[16]

2.2 CellSearch™

CellSearch™ works via immuno-magnetic cell selection. The majority of CTCs overexpress epithelial cell adhesion molecule (EpCAM) and can be targeted by anti-EpCAM antibodies immobilised on the surface of magnetic nanoparticles.[15] The CTC connected magnetic nanoparticles are then extracted from the blood sample using an external magnet, as shown in Fig. 2. The extracted cells were then put through immuno-fluorescent staining and observed under a fluorescence microscope. The main disadvantage of this process is the non-detection of non-EpCAM expressing CTCs.[16] CellSearch™ has shown to be expensive, time consuming and of variable efficiency on account. Nevertheless, this technique currently provides the better enrichment results compared to other selection methods comprised of several extraction steps. Hence, the need for alternative efficient separation processes which are simpler and cost effective.[16]

Flow cytometry is widely employed methodology for identifying cell subpopulations and is based upon the differential light scattering produced by cells of differing size and granularity.[25,26] Cells or particles are flowed through a laser beam and the relative light-scattering of each can be observed. Differentiation of cell types can be carried out based on difference in size and complexity allowing real time distributions to be observed. Cells can be isolated due to their fluorescence behaviour by addition of fluorochromes to the membrane, cytoplasm and nucleus or conjugated to antibodies.[27] Cell sorting allows sub-populations with desired characteristics to be separated by charging and differentiating electrostatically. Dependant on flow rates and sample cell concentration, thousands of cells can be sorted per second.[26,27]

A number of studies have aimed to capture CTCs by flow-cytometric based methodologies.[27–29] Lang *et al.* used EpCAM binding to target ten breast cancer cell lines from phosphate buffer saline (PBS) solution and spiked into PBMC mixtures to mimic CTCs in blood.[28] The study yielded variable results, with the highest retention being the MCF-7 cell line with 99.3% recovered from PBS, as well as a low retention of 0.002% for Hs578T cell line. This inability to target all cancers significantly limits the utility of the technique in a clinical setting. Furthermore, when delivered from a more complex PBMC suspension, the cell retention rates were substantially poorer and required additional sample preparation time. Lastly, the study also reported a rapid decline in cell retention as a function of time after collecting the blood sample, which makes it difficult to incorporate it into a clinical setting.[28]

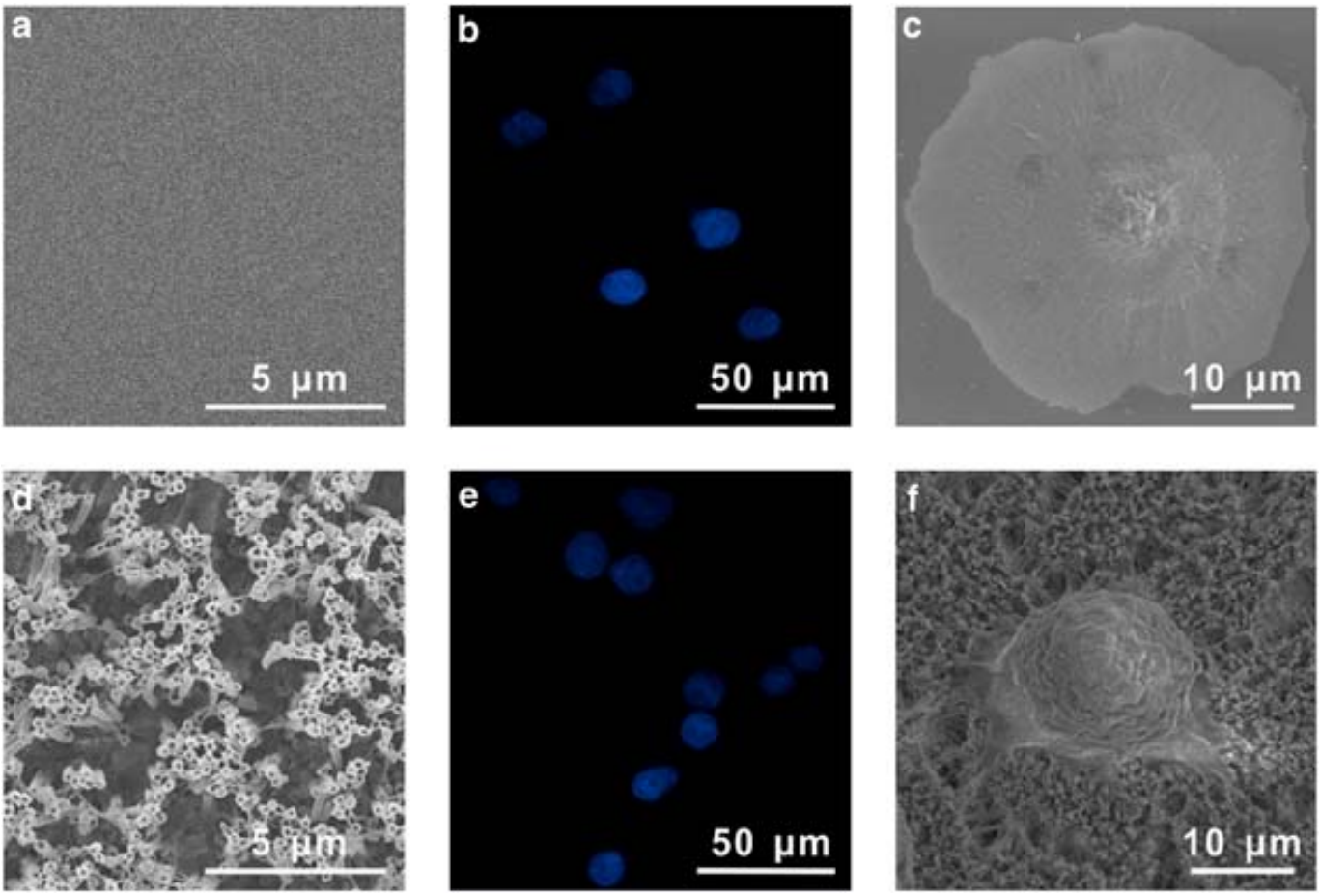

Fig. 4 Micrographs of a substrate-captured cancer cells showing the enhanced adhesion in case of modified PS NT substrate. (a,d) Scanning electron microscope (SEM) images of the smooth PS and PS NT substrates respectively. (b,f) Fluorescence microscope images of cells captured on these respective substrates. (c,f) Environmental scanning electron microscope (ESEM) images of cancer cell immobilised on these two respective substrates.[31] Reproduced from reference [31] with permission from [Nature Publishing Group], copyright [2013].

2.3 Antibody-based cancer cell recognition and capture

Molecular imprinting has been extensively used for the recognition and capture of CTCs using various binding agents in the form of antibodies, aptamers and peptides. Antibodies as binding agents have been widely reported compared to aptamers and peptides selection. Specifically, EpCAM has been targeted extensively for this purpose. Ovarian cancer cells (SKOV3) were successfully separated from whole blood in-vitro using centrifugation and electrochemical (eLOAD) integrated microfluidic device.[30] The anti-EpCAM antibodies were immobilised on gold electrode substrate using L-cysteine as the binding agent and the SKOV3 cells expressing the EpCAM bound to the gold electrode efficiently. The process optimisation resulted in a minimum capture efficiency of 87% and around 214 captured cells/mm^2 of the gold electrode surface.[30]

Anti-EpCAM antibodies were also used to modify a soft polystyrene nanotube substrate (PS NT) in order to detect and capture breast cancer cells. A BSA-Biotin (Bovine serum albumin-Biotin) conjugate was attached to the pillars using the hydrophobic interaction with further modification carried out using streptavidin, followed by biotinylated anti-EpCAM antibodies, as shown in Fig. 3. MCF-7 breast cancer cells attached efficiently to the anti-EpCAM antibodies on the pillars via the over-expressed EpCAM on its surfaces, as evident from Fig. 3b. Non-patterned surfaces showed a lower cell retention compared to those with protruding filopodia on the PS NT substrate.[31]

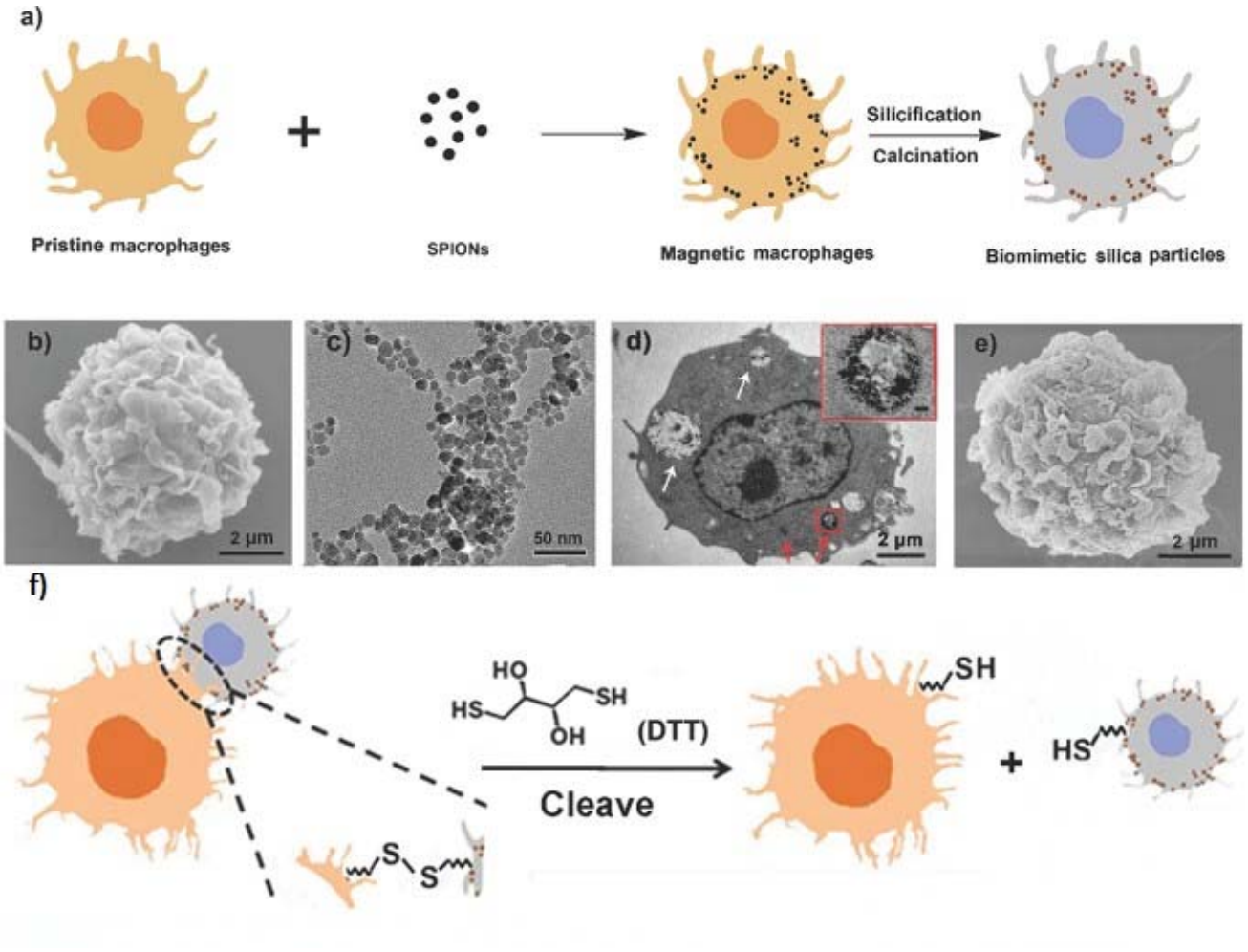

Fig. 5 (a) Schematics of the preparation of multifunctional smart particles using the live template strategy. (b) An SEM image of pristine macrophage. (c)-(d) TEM images of SPIONs and magnetic macrophage, respectively. White and red arrows indicate the SPIONs in vesicles or cytoplasm, respectively. Insert picture shows a higher magnification view of internalized SPIONs. Scale bar is 100 nm. (e) SEM image of the biomimetic silica particle. (f) Schematic showing the mechanism of cleavable disulfide bonds-based cell release.[32] Reproduced from reference [32] with permission from [John Wiley and Sons], copyright [2015].

Release of cells was controlled by decreasing the temperature to 20 °C, resulting in the transformation of the soft PS NT substrate to a hydrophilic state and henceforth the separation of the BSA-Biotin conjugate which finally is responsible for the release of the cancer cells.[31] The cells retained their viability due to the soft nature of the substrate and treatments. This method resulted in a capture efficiency of 95% of viable cells and could provide a promising way of surface modification for high-quality cancer-cell detection platform.

Figures 4a and 4d show scanning electron microscope (SEM) images of the PS and PS NT used to capture cancer cells, seen in fluorescence microscopy images presented in Figs. 4b and 4e. Cells retained to each substrate can be seen with environmental scanning electron microscopy (ESEM) images in Figs. 4c and 4f. Huang *et al.* also used the filopodias present on the surfaces of macrophages for the efficient separation of MCF-7 breast cancer cells from blood samples.[32] Smart particles were produced using the process of silicification and calcination of macrophages integrated with citrate-coated super-paramagnetic iron oxide nanoparticles (SPION), as shown in Fig. 5a. These multifunctional smart particles (MSP), which were silanised with biotinylated anti-EpCAM antibodies, were capable of capture and release of the EpCAM positive breast cancer cells. EpCAM antibodies were attached to the MSP using the streptavidin-modified disulphide linker and hence the attached cells can be released by inducing a cleavage in the disulphide linker, as shown in Fig. 5f.[32] The results of this study showed 50% capture efficiency along with 90% efficiency in the release of the captured cells.[32] In another approach, Lv *et al.* used photo-responsive immuno-magnetic nano-carriers for capture and release of CTCs.[33] Similar antigen-antibody interactions were used for the separation of the rare cancer cells from blood, but with the addition of a photo trigger in the form of 7-aminocoumarin, which acts as a connection between anti-EpCAM antibody and the magnetic beads, as schematically shown in Fig. 6a. The 7-aminocoumarin moieties overcame the C–O bond under both ultraviolet (UV) and near-infrared (NIR) light illumination, resulting in the release of the captured CTCs from the magnetic beads, as shown in Fig. 6d.

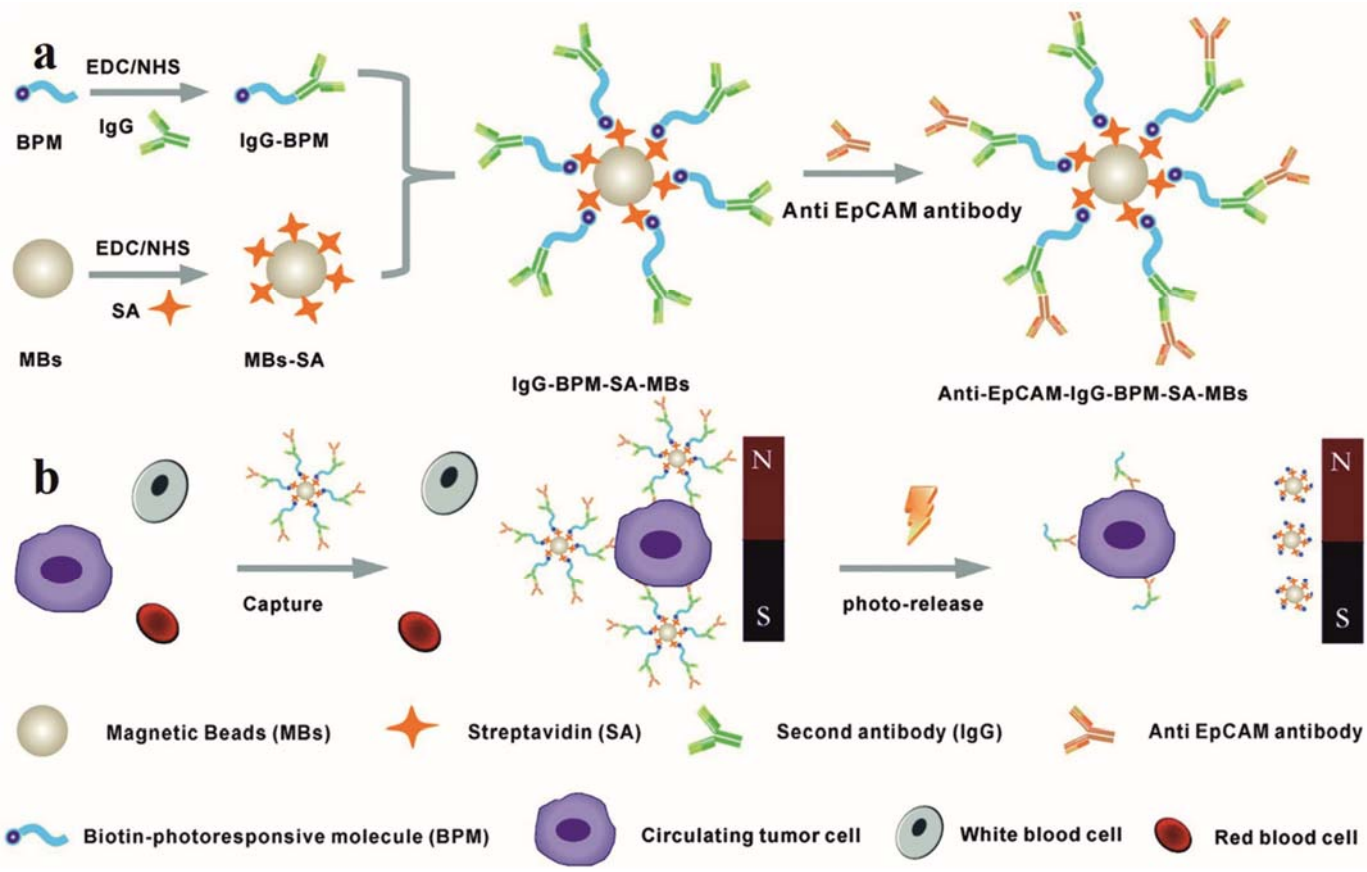

Fig. 6 Schematic showing the photo responsive immuno-magnetic system for capture and release of CTCs. (a) Production of the photo responsive immune-magnetic beads and (b) mechanism of capture and release of the cancer cells.[33] Reproduced from reference [33] with permission from [Royal Society of Chemistry], copyright [2015].

The whole process was carried out while preserving the viability of the cells involved. This specific technology resulted in 90% efficiency and 85% purity of the MCF-7 breast cancer cells. Under the irradiation of UV and NIR light, 73% and 52% of captured cells were released with a viability of 90% and 97%, respectively. This whole process was carried out on whole blood samples of cancer patients and hence opens a new route to cancer diagnosis and personalised therapy.[33]

The antibody-antigen interaction offers an attractive way for selective capture and release of specific CTCs, but at the same time the process is not cost effective and the availability is limited. In order to overcome these limitations, other natural receptors in the form of epitopes or aptamers (oligonucleotides) have been studied. The aptamers made of specific oligonucleotide sequences bind to different relevant proteins, microorganisms, cells and various chemical compounds. Aptamers made using the Systematic Evolution of Ligands by Exponential Enrichment (SELEX™) technology produce oligonucleotides with high target affinity. These aptamers were used extensively for the recognition of CTCs and ideally can be used as a replacement for antibodies in cancer diagnostics and therapies.[34,35] The aptamers unique interaction with their target comes from the specific 3D folding of the RNA or DNA oligonucleotides, which enables its recognition. Antigen-antibody isolation techniques suffer from disadvantages inherent to their biological nature; antibodies are expensive and have limited storage time. Moreover, not all malignancies have an immunophenotype amenable to specific targeting or which enables accurate differentiation of the malignant cells from normal cell populations. To overcome disadvantages associated with antigen-antibody interaction for CTC isolation, Zheng *et al.* developed a technique of screening specific CTC from a mixture of different cells using the so-called barcode particles coated with dendrimer amplified aptamer

probes.[36] The barcode particles were made using silica nanoparticles packed in emulsion droplets, which were used as templates. The evaporation of the solvent resulted in the formation of closely packed spherical colloidal clusters. The surfaces of these particles were then coated with dendrimers and DNA aptamer probes, as shown in Fig. 7. The DNA aptamers help in the detection and binding of specific target molecules on the surface of the cancer cells. The use of aptamers has many advantages over the use of anti-EpCAM in CTC detection.

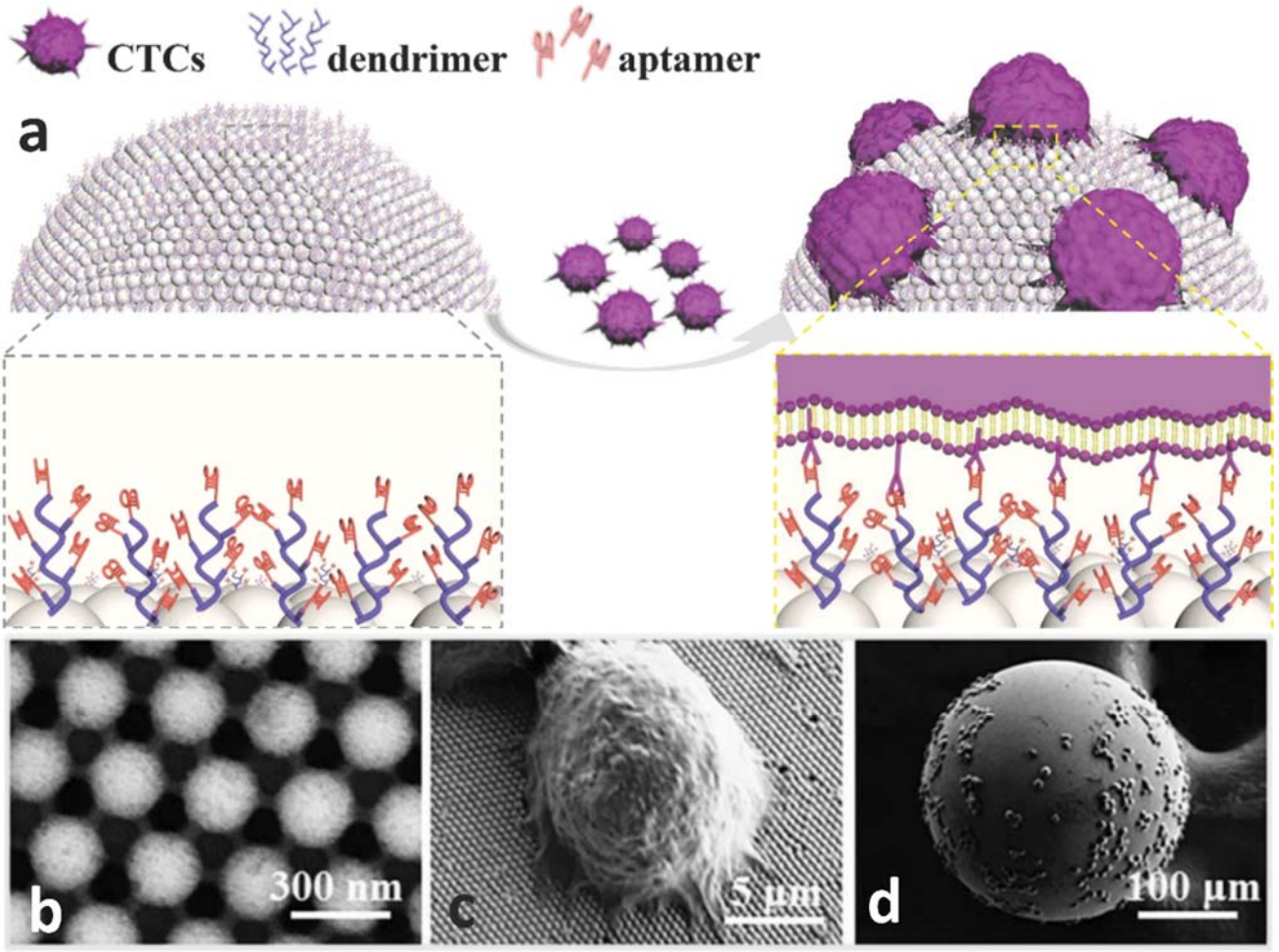

Fig. 7 Schematics and Field-emission SEM images of the barcode particles used for the detection and capture of CTCs. (a) Schematics showing the mechanism of the capture of CTCs using barcode particle surfaces modified with dendrimers and DNA aptamers. (b) FESEM images of individual barcode particles coated with the aptamers, (c) morphology and (d) distribution of the captured CTC on the barcode particles.[36] Reproduced from reference [36] with permission from [John Wiley and Sons], copyright [2014].

DNA aptamers are synthesised for very specific cell types and hence can differentiate between different cell populations suggesting a possible use of these techniques in cancer diagnostics. There are other advantages, such as lack of toxicity, less immunogenicity, higher stability and less blood residence time. An overall capture efficiency of 90% was obtained by Zhang *et al*. using DNA aptamers made for specific cells.[36] The barcode particles were modified using three different synthesised aptamers segment 'TD05', 'Sgc8', and 'Sgd5' for affinity towards Ramos (human lymphoma), CCRF-CEM (human leukaemia) cells and the last as control, respectively. The results showed a capture efficiency of 98% for Ramos and 97% for CCRF-CEM. Cell viability was maintained at 97% in the study. The use of exonuclease I resulted in the 86% release of the CTC from the barcode particles.[36]

In another study, specific antigen aptamers were synthesised and used for the detection of prostate tumour cells.[37] These prostate tumour cells express prostate specific membrane antigen (PSMA) on the cell surface that can be readily detected and captured using the specific antigen aptamers. The anti-PSMA aptamers were immobilised on the surface of a microchip made of polymethyl methacrylate (PMMA) and modified into a high throughput micro-sampling unit (HTMSU). The HTMSU was used for the identification of the prostate tumour cells from highly heterogeneous clinical samples (peripheral blood matrix). The HTMSU contains 51 ultra-high aspect ratio parallel curvilinear channels with a channel dimensions similar to that

of the prostate cancer cells. An extraction efficiency of 90% was obtained using this device for LNCaP cells. Captured cells were released using trypsin after separation. The HTMSU device incorporated a contact conductivity sensor in order to determine the number of captured prostate cancer cells upon release and hence there is no requirement for the staining of individual cells for quantitative analysis. The authors were able to separate prostate cancer cells from samples containing breast cancer cells, thereby demonstrating the specificity and sampling efficiency of the HTMSU device.[37]

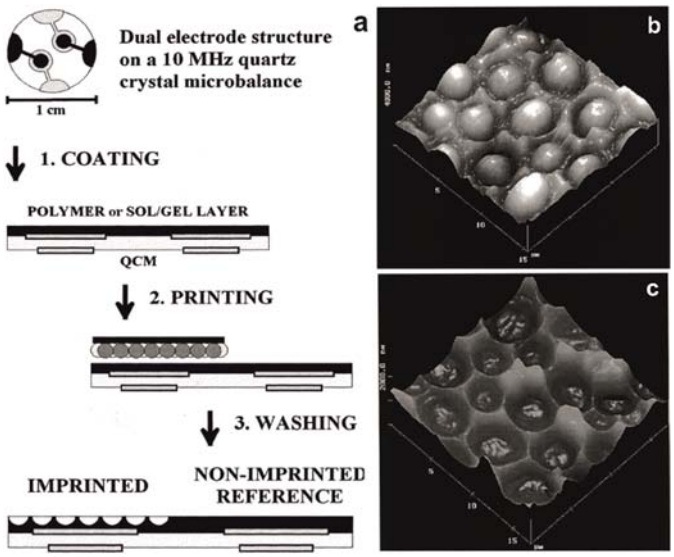

Fig. 8 (A) Schematic of the protocol reported by Dickert et al., to fabricate imprints onto the surface of quartz crystal microbalance electrodes. (B) Atomic Force Microscopy images of sol-gel layers from titanium (IV) ethylate whilst curing in contact with *S. cerevisiae* and (C) AFM images of cured bioimprint with densely packed layer of biomimetic receptors.[39] Reproduced from reference [36] with permission from [American Chemical Society], copyright [2002].

In another modification to the aptamer based cell cancer cell retrieval technology, Zhao *et al.* used multiple monovalent aptamer units on a 3D DNA network with size of over tens of μm in the solution.[38] The science was inspired from marine organisms with long tentacles containing multiple adhesive domains in order to capture food. This approach resulted in the enhancement of the leukemic cell capture and retrieval compared to the use of antibodies and monovalent aptamers. The 3D DNA network was created using the rolling circle amplification (RCA) method with specific control over the DNA sequence, graft density and length. The RCA aptamer immobilised on the DNA network binds specifically to the protein tyrosine kinase-7 (PTK7), which is overexpressed on different human cancer cell surfaces. This multivalent aptamer technology (along with herringbone microfluidic device) for cancer cell detection and separation significantly outperformed other monovalent aptamer antibody integrated microfluidic cell capture technologies.[38]

3. Bioimprinting

Bioimprinting is a promising approach for specific cell separation and targeting. Herein, polymer matrices are usually cured in the presence of a template material to yield cavities of a bespoke size, shape and orientation.[40–42] Fabricated artificial receptors are complementary to a desired cell topology and orientation and organisation of extracellular features; able to mimic the highly specific interactions, ubiquitous in nature.[43] Polymer-based imprinted systems are relatively cheap and are both mechanically and chemically robust, allowing prolonged storage life and reuse.[42,44] More importantly, a variety of materials can be used as the template, showing a great potential for the area.[45] The first bioimprinting studies were reported in the 1970's where substrates capturing the shape of templated macromolecules produced. The latter were then incorporated into chromatographic stationary phases, which were shown to vastly improve selectivity towards the original macromolecules.[46,47] Reports of a wide range of moieties were reviewed by Iskierko *et al.* including amino acids, carbohydrates and nucleotides bases.[45,47] Due to the arrangements of groups in space, imprinted materials have been shown to provide a method for enantiomeric separation of racemic mixtures. In doing so, Ansell *et al.* suggested molecular imprinting as a viable method for enantiomeric purification of drug candidates to overcome stringent legislation.[48] Success in the area was originally inhibited by intricacies in molecular structure such as high molecular weight, branching and variation in regions of charge or hydrophobicity.[40,44] For these initial studies, a bulk imprinting protocol was used whereby template materials were dispersed in the bulk of non-porous pre-polymers. After curing the matrix and grinding the resultant system into beads, the template could then be chemically removed and the beads sieved to achieve a desired diameter.[40,42] However, little control of the degree or uniformity of grinding can be achieved and the quality and reproducibility of imprinted cavities varies significantly.[42] Also, as analytes increase in size and complexity, it becomes more difficult to elute samples over the imprinted surface.

3.1 Whole cell bioimprinting

The landmark publications by Dickert *et al.* showed the first examples of cell surface imprinting techniques.[39,66] These studies described a surface micro-contact imprinting technique that was able to express the morphology of yeast cells in sol-gel surfaces. Yeast cells were immobilised to glass substrates in order to form a cell 'stamp' which could then be pressed into a curing sol-gel material. Once polymerised, the imprint was removed, washed and analysed via Atomic Force Microscopy (AFM). The imprint was shown to yield a densely packed, regular honeycomb lattice of cell cavities in a complementary shape to the template yeast. As template cells were not fully immersed in the matrix, the problems associated with uneven grinding and sample elution are negated. The sol-gel material was reported as an ideal imprinting matrix due to the ease of formulation and resistance to scratching. Furthermore, by imprinting quartz crystal microbalance (QCM) electrodes, the study was able to accurately test the retention of cells back on to the imprinted surface. When incubated with several strains of yeast, imprinted sol-substrates showed a high affinity to the target cell type with on-line monitoring. Figure 8a shows the

schematic of the protocol used in the study and Fig. 8b presents an AFM image of one of the imprinted surfaces. These authors followed up on their pioneering study by expanding the range of template materials captured into bioimprints such as enzymes and viruses.[52,53] They proposed a method to screen complex matrices for viruses, in this case tobacco plant sap, circumventing time-consuming sample preparation. Substrates were analysed in real time by using QCM analysis.

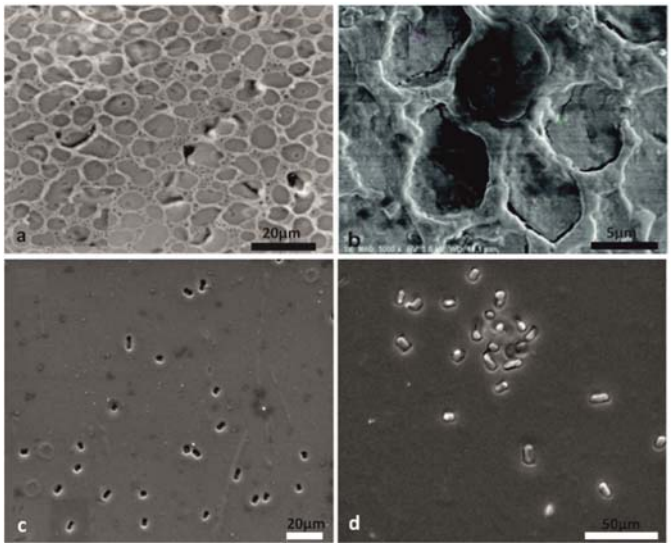

Fig. 9 (a) Contact mode AFM image of pollen imprinted polyurethane,[49] (b) SEM image of algal cell imprint captured on poly(ethylene-co-vinyl alcohol) film[50] and (c) images of *cryptosporidium parvum* oosysts and (d) adsorption of new oocysts in suspension after incubation with the imprint.[51] Reproduced from reference [49,50,51] with permission from [Springer-Verlag, Royal Society of Chemistry, MDPI], copyright [2009, 2014, 2010].

Imprinted films selectively captured micro-organisms from aqueous suspensions with high adsorption affinities, showing the potential for their inclusion in biosensors.[54] Following these studies, a wealth of whole cell imprints have been recorded using spores, yeast, bacteria and multitude of mammalian cells. Jenik *et al.*[49,55] reported a surface imprinting technique using two different pollen grains into polydimethylsiloxane (PDMS). Using a QCM sensor, they were able to show in real-time the selective uptake of pollen by the biosensor device. Though nanoscale printing of macromolecules and even whole cells had been achieved, this study showed bioimprinting to be effective at targeting comparatively large analytes; pollen grains vary in size between 10-50 μm[55] (see Fig. 9a), similar sizes to those of cancer cells. Cohen *et al.* reported the successful imprinting of various strains of bacteria into sol-gel films.[56] Bacteria with differing morphologies and extra cellular features were incubated on imprinted substrates and the retention analysed by a combination of AFM, SEM and confocal laser scanning microscopy (CLSM). The study noted a 90% affinity to the target organism type due to their distinct 'macromolecular fingerprint'. Figure 9c shows SEM images of bacterial bioimprints and Fig. 9d shows bacteria retained to imprinted surface after incubation. Lin *et al.* reported surface imprinting and recognition of algae cells into poly(ethylene-co-vinyl alcohol) films (see 9b).[50] These authors were able to examine the algal cell metabolism and their hydrogen production by incorporating bioimprints on biofuel cells. Fuel cells showed increased output, likely due to the increased expression due to the artificial microenvironment created.[50]

Recently, Bao *et al.* reported a new approach, where a bacterial imprint on polymer of outer surface charge heterogeneity was synthesized which demonstrated highly specific bacterial recognition.[57] The charge distribution on the outer surface of the bacterial cells was captured by the bioimprint during polymerization by the self-assembly of the two different monomers around the template (see Figs. 10a-10c). Subsequent covalent binding of this templated monomer arrangement into the bioimprinting matrix created chemical imprints for bacterial recognition. These authors demonstrated that by using this novel approach, target bacteria can be preferentially captured due to stronger and cell shape-specific electrostatic interactions (see Fig. 10d). They emphasize the versatility of this fabrication strategy since a variety of charged monomers could be exploited as building blocks in surface-initiated atomic transfer radical polymerization. This approach can also be extended to recognize bio-macromolecules or other biological entities associated with distinctive charge distribution.[57] We envision that similar approach could become a versatile platform for developing biological recognition materials for cancer cells.

4 Applications of cell bioimprinting

4.1 Biosensors

Biosensors are bioanalytical instruments containing a specific recognition entity coupled with a physiochemical transducer.[10,58] In a clinical setting, biosensors provide rapid diagnoses with small sample size. Lowering the limits of detection for such devices is of importance as early discovery and treatment of disease can improve patient prognosis, particularly in cancer. Once isolated, tumour markers such as CTCs can undergo phenotypic and genetic tests to assess patient prognosis and tailor treatment.[10,11] Although the bioimprinting as a technique has started by imprinting of simple molecules and rudimentary microorganisms, recent studies have incorporated a range of human cell templates for bioimprinted substrates. The inclusion of such substrates into biosensor design can provide discrimination between cells of very closely matched properties.

Dickert *et al.* were able to create a biosensor device for ABO blood grouping. By using erythrocytes of the blood groups A, B, AB and O, they produced bioimprinted layers of polyurethane. Erythrocytes of different blood groups are morphologically identical and differ only by varied surface antigens. Therefore, the selectivity reported is reliant only on hydrogen bonding between interaction sugar residue antigens and the bioimprinted surface.[60] They characterised the selectivity of substrates imprinted with erythrocytes of blood groups A, B, AB and O by incubating cells of each type. Although the use of cell imprints gave mixed results, there was a clear preference to the

blood group used to template the bioimprints. Selectivity experiments were initially carried out in a buffer solution but they also went on to use whole blood. Although in this case they registered lower cell sensitivity, it was demonstrated the viability of bioimprinted substrates in use with whole blood samples with very little additional sample preparation. A subsequent study was able to discriminate between subtypes of erythrocytes of blood group A, known as A_1 and A_2.[55] The publication describes the retention of erythrocytes to be dependent on type, abundance and orientation of cell membrane antigens.

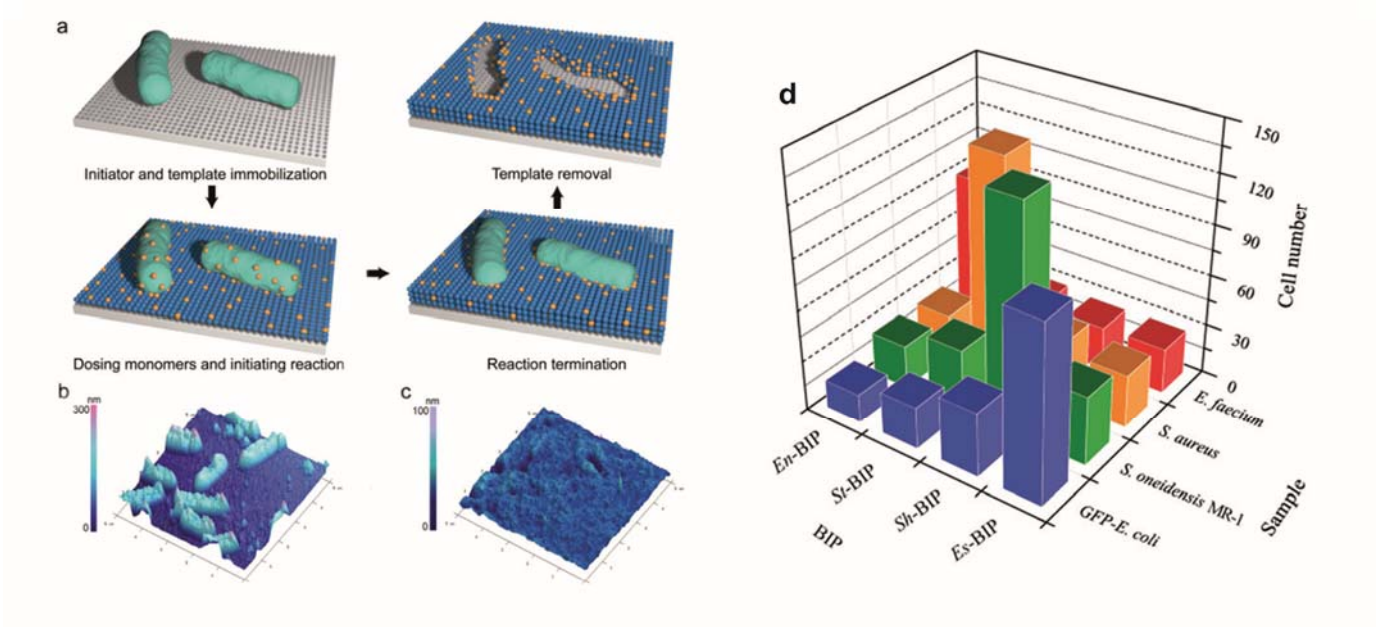

Fig. 10 (a) Schematic diagram of the bioimprint fabrication process with surface charge heterogeneity. AFM images of the polymeric bioimprint before (b) and after (c) removal of the *E. coli* template. (d) Numbers of different bacterial cells captured on the bacteria-imprinted polymer (BIP) imprinted with different cell templates. [57] Reproduced from reference [57] with permission from [Royal Society of Chemistry], copyright [2017].

Bioimprints are able to discriminate analytes on a nanometre scale identical in overall size and curvature. Retention of the other subtype of erythrocyte was found to be negligible; similar to cells retained to non-imprinted substrates. The ability of bioimprints to discriminate between cells identical on a micrometre scale, due to differences in nanometre scale signifies possible applications as biosensors, separating cells based on subtle differences in cell-surface proteins.[55] We envisage that such differences may be important in the development of tools for bioimprint based cancer diagnostics.

Eersels *et al.* combined cell surface imprinting (SIP) with heat transfer resistant measurement in order to detect human cancer cells and macrophages.[59] The entrapment of the cells in the cavities of the SIP layer resulted in the significant increase in thermal resistance at the solid-liquid interface. This property was used to detect the immobilisation of cells derived from the immortalised cell-line ZR-75-1, on the printed polyurethane substrate. ZR-75-1 cells were spread and allowed to sediment on a PDMS substrate forming a dense monolayer coverage. The cells morphology was captured in polyurethane resin layer by pressing the cell-adorned PMDS stamp against a curing polyurethane surface. AFM was used to analyse the topology of the imprint surface. The imprint is mounted on a copper base and heated to a constant temperature (T_1=37 °C) with the temperature of the cancer cell effluent (T_2) monitored. Figure 11 represents the schematic of this experimental setup.[59] Taking into account the power used to heat the imprint surface, the thermal transfer resistance was calculated as cell adhered to the imprint. The study reported specific binding of cells to the imprinted cavities, which noticeably increased the thermal resistance at the solid-liquid interface.[59]

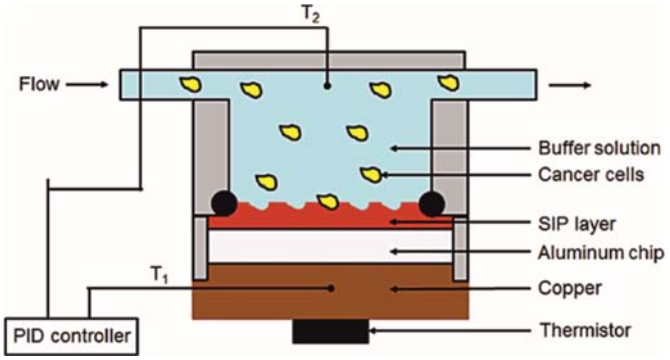

Fig. 11 Schematic view of the device used to detect uptake of target macrophages to bioimprinted surfaces by thermal resistance measurement by the heat-transfer method (HTM).[59]

Reproduced from reference [59] with permission from [John Wiley and Sons], copyright [2015].

Eersels *et al.*[60] reported MCF-7 breast cancer cell imprints which were characterised using the optical microscope, shown to have an average cell diameter of 20 µm (see Fig.12a). This agreed with the AFM analysis image of the polyurethane imprints Fig. 12b). The results showed the selective nature of the imprints with 15-20% imprint response compared to 1.4-5.2% of cross contamination from non-imprinted cells. The gradual rinsing of the imprint resulted in significant decrease in cross-contamination from non-imprinted cells as evident from Fig. 12c, 12d and 12e indicating an increased adhesion to the target cells. The application of excess forces by rinsing was adequate to eliminate any non-specifically attached cells.

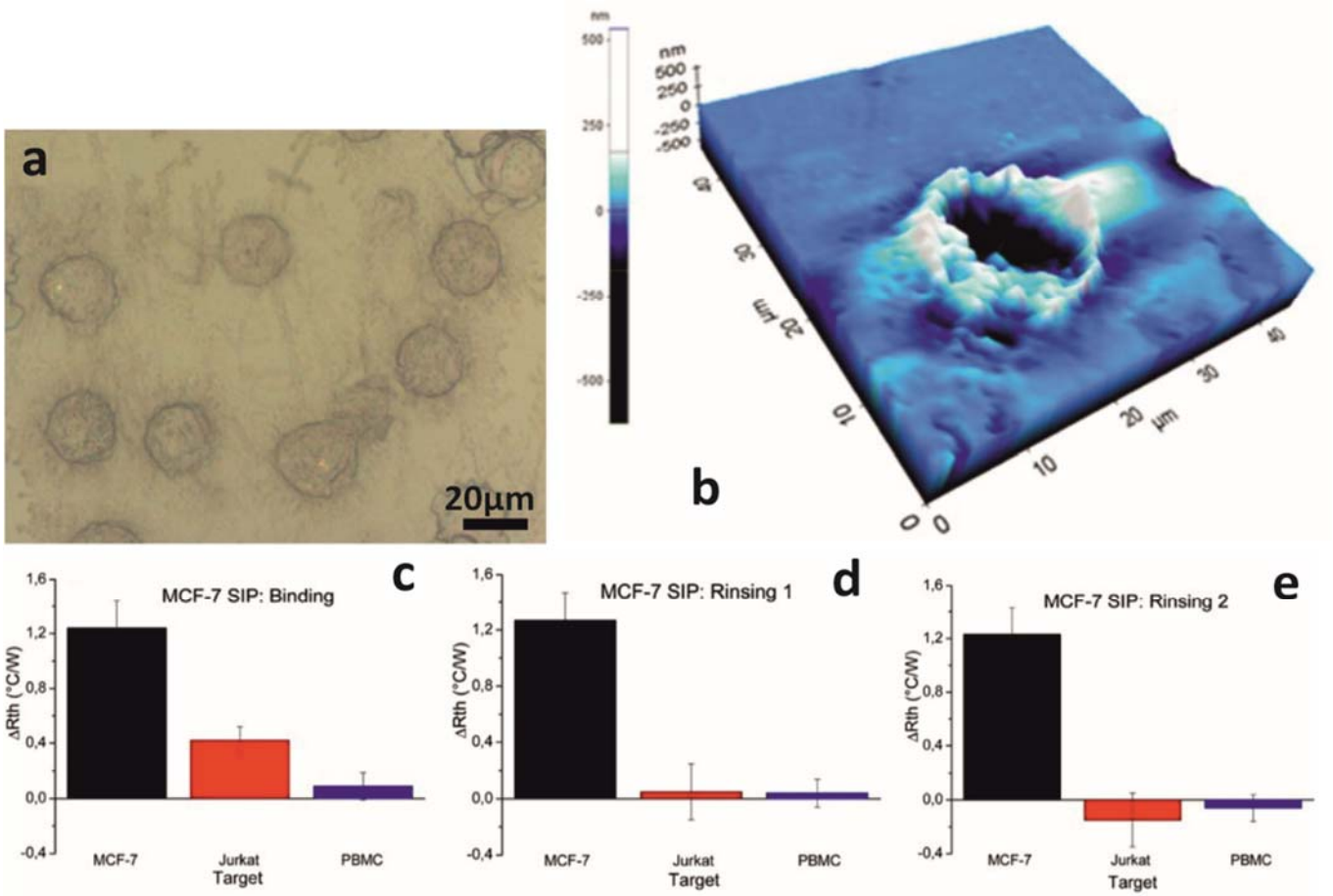

Fig. 12 (a) Optical microscope image of the surface imprinted polymer (SIP) for MCF-7 breast cancer cells, (b) 3D representation of the AFM of a single MCF-7 imprint on polyurethane, graphical representation of change in thermal resistance ΔR_{th} of MCF-7 SIP upon exposure to MCF-7 cells with attachment (c) and (d) and (e) consecutive rinsing steps.[60] Reproduced from reference [60] with permission from [American Chemical Society], copyright [2013].

Zhang *et al.* reported the *in-vivo* targeting of CTCs to circumvent limitations associated with standard *in-vitro* blood tests.[24] Herein, nylon substrates were functionalised with anti-EpCAM antibody via 3-aminopropyltriethoxysilane (γ-APS) and carboxymethacrylate (CBMA) coupling. Uptake of CTCs onto the biomimetic device was examined and the results showed an affinity to tumour cells expressing EpCAM. The biocompatibility of nylon as a substrate was also reviewed and shown to be suitable for *in-vivo* assays. The authors suggest this method will overcome problems in sample size attributed to blood harvesting or negate the need for further culturing of isolated cells.[24] Both could vastly improve the diagnosis of cancer. Moreover, by optimisation of the experimental apparatus and the methodology sensitivity could be improved with the limits of detection lowered.[24] In an effort to target cancers not expressing EpCAM, Jackson *et al.* used a microfluidic device with combinations of other antibodies grafted into a polymer matrix.[23] A microfluidic device was made comprised of three chambers each targeting a different antigen expressed by acute myeloid leukaemia cells (AML), namely CD33, CD34 and CD117. Using immunohistochemistry, these authors were able to identify each cell type retained to the imprinted substrate. When applied to samples from AML patients in remission following successful therapy, the microfluidic method showed vastly superior sensitivity to standard bone marrow and blood aspirate analysis. Moreover, the method was able to detect relapse in a patient 57 days after stem cell transplant compared to the 85 days with conventional diagnostic approaches.[23]

Blood and bone marrow analysis are invasive and painful.[23] Additionally, not all types of leukaemia have phenotypes amenable to monitoring for possible relapse after the completion of therapy. Due to the smaller sample volumes and processing time, the group predict the device suitable for >90% of patients with smaller intervals between each. Due to the versatility of the method, the microfluidic device can be used to detect the various types of leukaemia and lymphomas.[23]

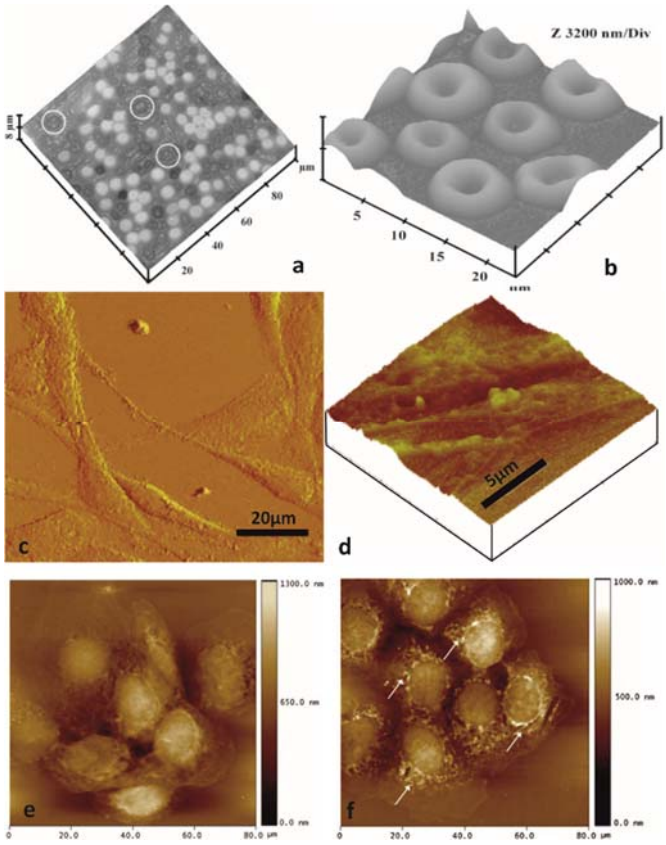

Fig. 13 AFM images of (a) erythrocyte imprinted polyvinylpyrrolidine with cavities highlighted. Cells are also seen retained from the imprinting process. (b) Artificial erythrocyte made from subsequent imprinting procedures.[55,60] (c) Imprint showing the muscle cell surface characteristics and (d) angular view of imprint showing muscle cell membrane roughness.[67] AFM images of Ishikawa endometrial cancer cell bioimprint in (e) native conditions and (f) cancer cell replica made from cells exposed to $CoCl_2$ to induce their pores to open.[64] Reproduced from reference [55,67,64] with permission from [Elsevier, AIP Publishing LLC, Elsevier], copyright [2009, 2009, 2011].

4.2 Nanoscale cell surface analysis

Protocols to analyse cell topologies with both scanning electron and transmission electron microscopy (SEM and TEM) are commonly used, however, such methods do not achieve a sufficient resolution for nanometre scale analysis.[51,62,63] Difficulties using AFM are also well documented, with a high pressure applied to cell membranes via the scanning cantilever, irreparable damage can be caused to the cells of the living tissue. Moreover, the deformation or the movement of living cell membranes can lead to imaging results that are not truly representative of the cell morphology.[64] AFM has been used to analyse both negative and positive bioimprints as an alternative to direct cell imaging.[65] Negative bioimprints involve a replica of the original cells or membranes while positive bioimprints are casts of the respective negative bioimprints.

Muys *et al.* immobilised living rat pituitary cells and captured their morphology into a polymer matrix.[63] After curing the polymer, the topology of the bioimprints was analysed by tapping mode AFM, which showed cell shapes with no evidence of dehydration of the cells. Membrane pits and depressions were seen on the surface of the imprinted cells, consistent with those used for cellular exocytosis.[63] Similar results were achieved by the group using human endometrial cancer cells as templates. Bioimprints can potentially circumvent problems in analysing fragile biological samples using positive imprints as proxy cells. Analysis of extracellular membrane features on nanometre scale can yield information on cancer cell signalling and proliferation.[63]

Samsuri *et al.* expanded the templating of endometrial cancer cells, see Figs. 13e and 13f. They were able to correlate numbers of cellular membrane pores on the bioimprints with the quantity of vascular endothelial growth factor (VEGF) excreted from cells.[64] Both authors[63] were able to achieve nanometre scale resolution of extracellular structures on endometrial cells, able to be analysed by AFM.[64] Samsuri *et al.* also targeted mammalian cells. By using a UV curable biocompatible matrix that has rapid yet ambient cure times, they successfully bioimprinted live human muscle cells. Figs. 13a and 13b show AFM analysis of erythrocyte imprints.[55,60] Upon AFM analysis, microstructures and cell membrane features were imaged with nanoscale resolution. Processes such as neurotransmission, enzyme secretion or hormone release can be attributed to nanoscale transformations on the cell membrane.

The ability to characterise variations in structure and morphology of cell membranes may be useful in the diagnosis of malignancies and other diseases. Moreover, membrane abnormalities on a molecular level may be useful for the diagnosis and prognostication of a variety of solid and haematological tumours.[62,64]

4.3 Cell culture platforms

Extracellular environments have been shown to influence the growth of dividing cells.[68–70] Reports have detailed the use of bioimprinted substrates to mimic *in-vivo* growth conditions in order to study the proliferation of cell lines. The progression of cancers can be monitored in environments representative of the body.[71] In particular, the effect that topographical mechanical signals have on the progress and action of any adherent cell lines can be classified.[72,73] Bioimprints offer a cheap and simple route to fabricate bespoke cavities on durable polymer substrates on which the cell growth can be stimulated. McNaughton *et al*. imprinted immortalised human cell lines: human cervical cancer (HeLa) , human kidney (HEK-293) and human lung (MRC-9) into various polyacrylamide hydrogels.[70] The various cell types were incubated aseptically on each

individual bioimprint surface. Dense cell growth was noted confined to the imprinted areas of the substrate.[70] The study showed that the imprint surface cavities act as docking sites to promote specific adhesion of cells and their growth.[70]

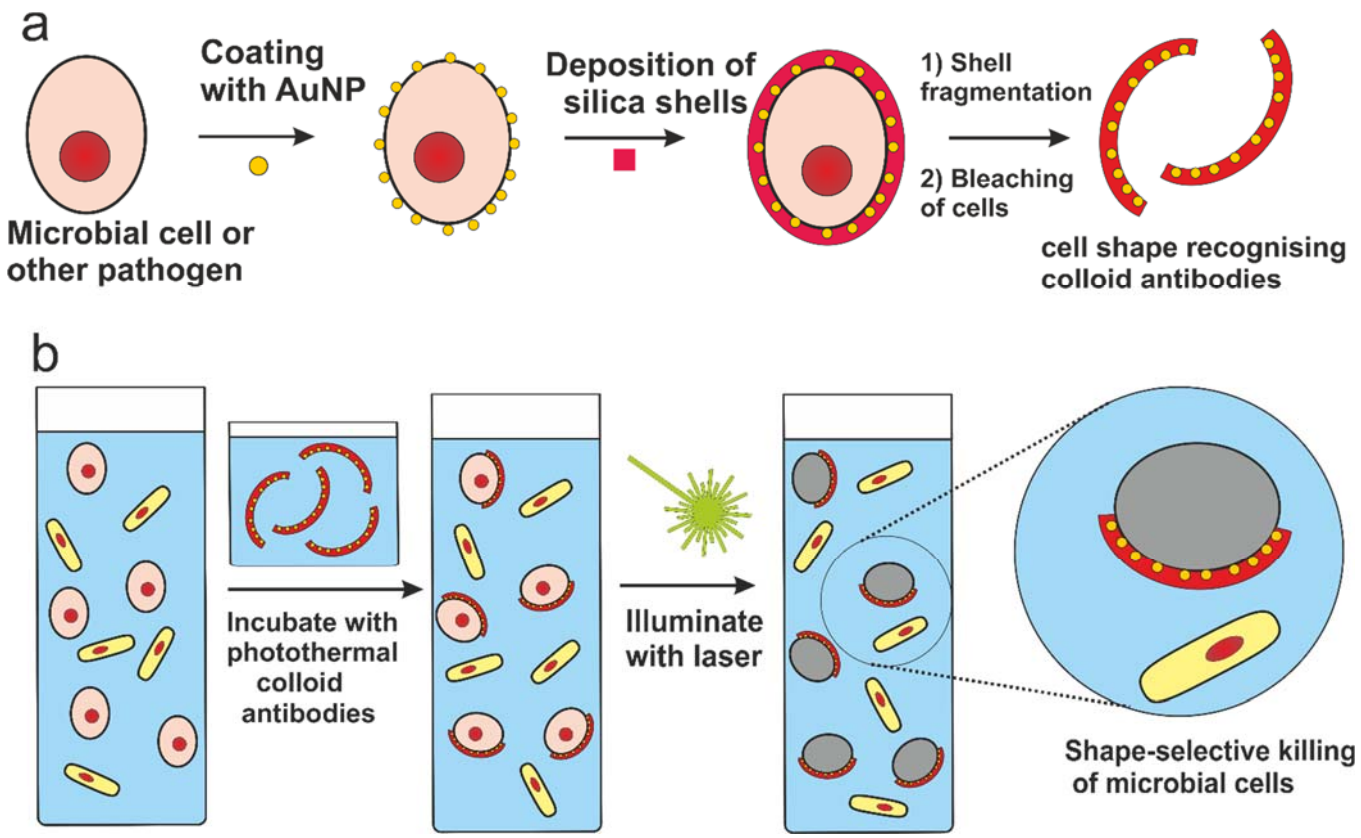

Fig. 14 (a) Schematic of the production and action of colloid antibodies reported by Paunov *et al*. where (b) explains the action of the photothermal killing of cells to use in conjunction with colloid antibodies. [79] Reproduced from reference [79] with permission from [American Chemical Society], copyright [2013].

This was also shown by Murray *et al*.[73] Jeon *et al.* imprinted fixed osteoblast-like cells (MG63) on a PDMS matrix.[68] MG63 cells were cultured on the surface of imprints and various cell activities, like cell viability, alkaline phosphate (ALP) activity and mineralisation were monitored. These results showed that imprinted substrates facilitate cell metabolic activities when compared with smooth culture substrates.[68]

Vigneswaran *et al.* proposed bioimprinted substrates to be scaffolds for the development of tissue engineering technology.[69,74] The studies both imprinted Ishikawa endometrial cancer cells into a UV fast-cure polymer. The results replicated studies showing the topology of the bioimprint to be representative of template cells on a micro and nanoscale. The authors suggest that bioimprinting can be used as a novel tool to improve understanding of the proliferation of cancer cells, vaccine preparation and other drug studies.[69] The study proceeded to characterise the ideal properties of scaffold used in implants, and demonstrates the promise of such bioimprinting approach on cell growth.[74]

Tan *et al.* also report the imprinting of Ishikawa endometrial cancer cells, in this case using polymethacrylate (pMA) and polystyrene (pST).[71] The studies compared a culture of cells on each bioimprint type and compared them to those on flat (non-imprinted) substrates. Characteristics such as morphology, cell-responses and antigen expression were monitored by AFM analysis of the surface of positive bioimprints. These studies demonstrated an increased proliferation of endometrial cancer cells on pMA, producing cells with a larger average diameter than cultured on flat substrates or pST. On both imprinted materials cells showed an increased expression of β-1-integrin, focal adhesion kinase and cytokeratin-18. The study shows the microenvironment in which cell are cultured modulates cell signalling and ultimately their proliferation.[71] Monitoring of the cancer development in such *in-vitro* environments allows for an improved understanding of how the condition progresses in patients.

Bioimprints have also been used to examine the effect of chemotherapeutic agents on cancer proliferation. In another study, Tan *et al.* produced imprints of endometrial cancer cells into polystyrene.[75] They showed that by culturing Ishikawa cells on imprinted substrates, the effect of chemotherapeutic agents could be evaluated on caspase-3-expression, proliferating nuclear antigen (PCNA) expression, VEGF (vascular endothelial growth factor) secretion and overall cell numbers. Their study examined how the physical environment modified the sensitivity of cells to treatment. It was found that positively and

negatively imprinted platforms were preferred by different chemotherapeutic agents when administered in single doses.[75]

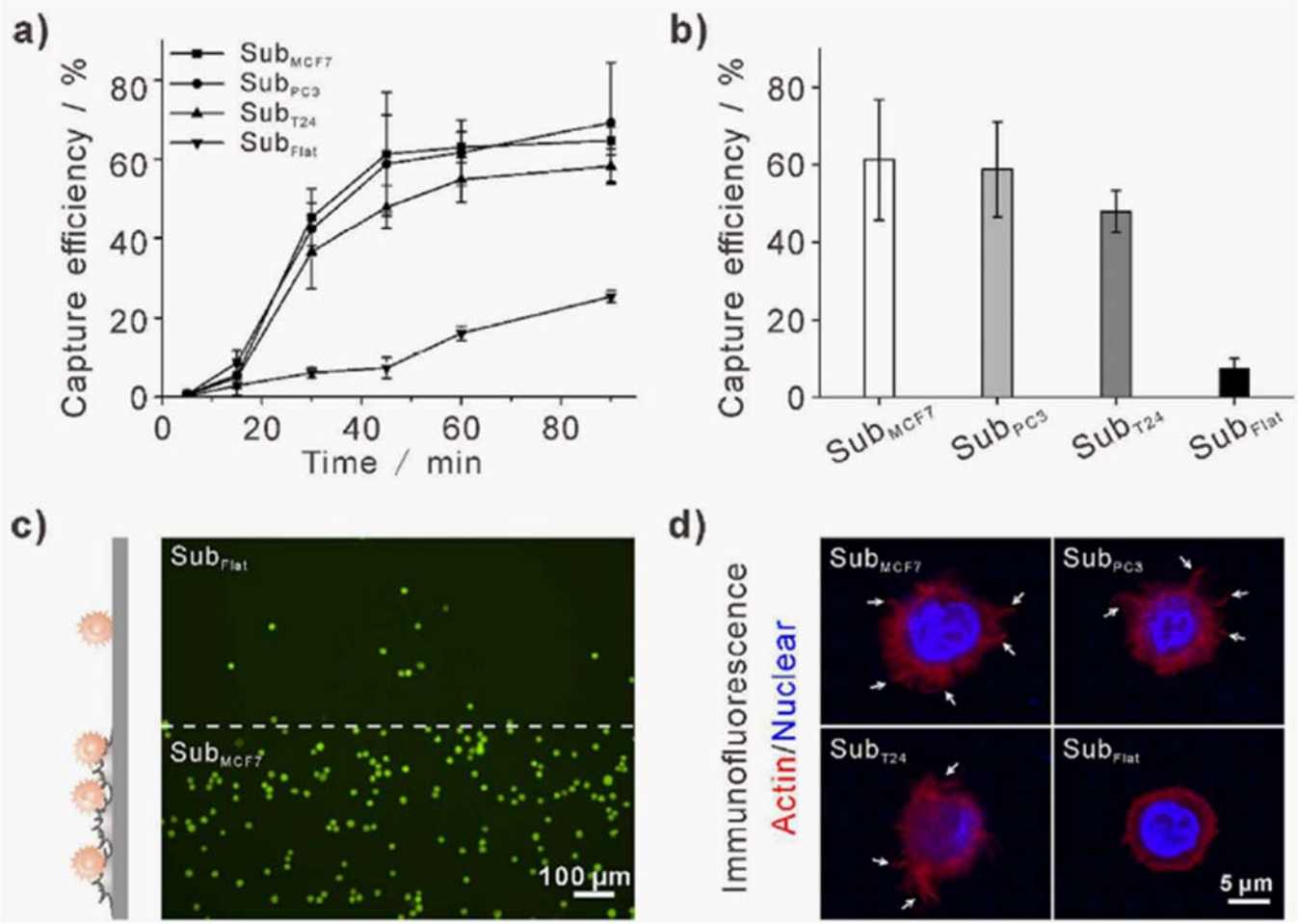

Fig. 15 Evaluation of MCF-7 cells capture performances on the as-prepared cell replica surfaces after anti-EpCAM modification. (a) With the increase of incubation time, the capture efficiencies of MCF-7 cells increase significantly and reach a maximum value around 45 min. (b) In comparison with other surfaces, SubMCF-7 and SubPC3 show higher capture efficiencies of MCF-7 cells at incubation time of 45 min. The capture efficiencies of MCF-7 on cell-replicated surfaces are much higher than that on the anti-EpCAM modified flat glass. (c) A fluorescent image of captured MCF-7 cells on SubMCF-7 and SubFlat in a close experiment setup. (d) Immunofluorescence images (actin, red; nuclear, blue) of captured MCF-7 cells on different surfaces. MCF-7 cells own more protrusions on SubMCF-7 and SubPC3, while exhibit less protrusions on the other two surfaces. Arrows indicate the protrusions of MCF-7 cells.[84] Reproduced from reference [84] with permission from [American Chemical Society], copyright [2017].

4.4 Drug delivery from bioimprinted particles

As mentioned previously, the main drawback of current cancer therapies is a reliance on non-specific targeting of any dividing cells. By the modification of the scheduling and dosage of chemotherapeutic agents, clinicians attempt to maximise the cytotoxic effect of the treatment of malignancies to an extent that is safe for healthy tissue. So far improvements in therapy have shown limited success. Higher remission rates have been achieved using high dose chemotherapy, however this remains unsuitable for the majority of patients due to poor prognostic factors such as old age and comorbidity.[1-3] Bioimprinting presents a methodology which could introduce selectivity to chemotherapeutic treatment. If cytotoxic materials can be focussed directly on cancerous cells, higher doses could be delivered to the malignant tissues with few side effects.[76–78] The vast majority of drug delivery vehicles reported are as functionalised particles, imprinted with recognition entities.

Colloid antibodies for cell shape recognition and targeting were reported by Paunov *et al.* [79–81] (see Fig. 14). The cells were further coated with silica, which after fragmentation and bleaching, yielded partial shells with an interior void of the complementary shape to target cells. When immersed in cell suspensions, colloid antibodies showed high selectivity, binding the target microbial cells in a suspension with other cells of different shape and size.[78] The authors expanded the study to

selectively kill target cells by embedding gold nanoparticles to the inner side of colloid antibodies.[79-81] In this study, the membranes of target cells were functionalised with gold nanoparticles, which were further encased in the silica shells along with the whole cells during the templating process. After fracturing the silica shells with ultrasound and bleaching the cell templates they produced 'photothermal colloid antibodies', where the silica hemi-shells matching the shape of the target cells had gold nanoparticles on their inner side (see Fig. 14a). Such photothermal colloid antibodies were then used to selectively bind to the target cells in a cell mixture and the whole sample irradiated with a laser. Due to the localized heating around the gold nanoparticles, the authors demonstrated that they can specifically kill only the shape-matching target cells. There is scope for cancerous cells to be used as the target for such studies to selectively ablate malignancy.[79–81] Liu *et al.* also produced functionalised nanoparticles (NPs) to exhibit a selective action on cancer cells. The study targeted malignant tissues on account of over-exposure of polysaccharides on the cell membrane sialic acid; a universal feature of cancer cells. Nanoparticles imprinted with monosaccharides showed an affinity to the specific monosaccharides expressed on the cancerous cells surface. In their study the NPs were doped with a fluorescent tag to improve selective imaging of malignancies. However, these authors speculate that with minor adjustment, the technique would be suitable in probes for targeted photothermal therapy for cancer.[82]

Doyle *et al.* also attempted specific targeting via hydrogel microparticles.[83] They aimed to build on previous findings to optimise particle design for cancer therapy. For instance, the flexibility of the particles dictates the overall circulation around the body and therefore the bioavailability. Microparticle shape has also been investigated with rod-like particles showing increased uptake by cancer compared to more spherical species. Various shapes of hydrogel microparticles functionalised with anti-EpCAM were fabricated. By systematically changing the particle shape the study was able to characterise the effect of surface area, hydrodynamic effects and steric effect uptake of particles to cells. Breast cancer cells (SKBR3s), which express EpCAM, were used to confirm the uptake of the octopus-like shaped hydrogel microparticles. Cells not expressing EpCAM (SKMEL28s) were also monitored and shown not to adhere to the microparticles. Specifically, the study showed that octopus-like shaped microparticles offered the best morphology for cell capture due to the heightened surface area contact.[83]

Wang *et al*. investigated the topographic interactions between three MCF-7 cancer cell lines with differentiated morphologies and their imprinted replica surfaces.[84] They demonstrated two levels of topographic interactions between cancer cells and their replica surfaces. The nanostructures on templated surfaces led to structural matching between nanoscale components on the cell surface and these nanoscale structures on the imprinted substrates. They report that in addition to the nanostructure, the microscale topography also enhances the topographic interaction between the cancer cells and their replica surfaces by the trapping effect — see Figs. 15a and 15b. These authors conclude that bioimprints that replicated multiscale structures exhibit improved affinities with cancer cells by synergistic effect of cooperative topographic interactions and molecular recognition which shows much higher capture efficiency compared to flat substrates. The suggested explanation is that the cell recognition is enhanced by the protrusions from the cell surface and their imprints (see Figs. 15c and 15d). This approach is likely to advance the smart design of multiscale bioimprints with highly specific cell recognition and provides an alternative to investigate interfacial properties of the cancer cells. [84]

Conclusions and future outlook

In this review, attention has been focused on comparing the different approaches for separating cancerous cells from complex cell suspensions. Current techniques rely on exploiting differences in chemical and physical properties of cells. Bioimprinting techniques can allow improved understanding, diagnosis and selective depletion of neoplastic cells. Antigen-antibody interactions have often been exploited mainly by targeting EpCAM, which is commonly expressed on cancer cells. EpCAM discrimination has been explored in a number of conformations, and is the cornerstone of current cancer cell targeting. EpCAM studies have been shown to be expensive, have limited storage life and are time intensive. Most importantly, neoplastic cells offer a poor target as the target antibody is not expressed unilaterally on all CTCs.

The field of molecular imprinting has been highlighted as a route to afford selectivity toward cancer cell recognition. Polymer matrices are functionalised with micrometre- and nanometre-scale features, complementary to target molecules, motifs and/or whole cells. Reports have shown substrates to be preferentially retained to bioimprint surfaces from multi-cell type suspensions. Successful studies based on recognition of bacteria make similar approach to specific cancer cells particularly promising, although requiring significant differences of cell types in the mixture. However, high affinity of cell-bioimprint has been seen in less heterogeneous mammalian samples. In particular, the blood grouping study shows the ability of bioimprinted substrates to distinguish among closely matched cell types. Bioimprints can discriminate cell types on account of the orientation and abundance of nanoscale features, not just the cell microscale size and shape.

Bespoke fabrication to target cell types is a key advantage of bioimprint design. Target cell types do not need to differ significantly from healthy counterparts or express particular extracellular features.

An inherent problem exists of sourcing large quantities of target cells in order to produce the larger scale bioimprints required for such a cell separation device but can work on small scale for biosensors. This limitation, however, should be able to easily be overcome. Once an initial positive bioimprint stamp is formed from a reduced number of cells, this stamp can be repeatedly imprinted into a larger surface. Despite the need for some repetition in an imprint formed in such a way, this should allow

replication of the original bioimprint on a much larger scale without the need for further cell culturing.

Although studies have showed the successful imprinting of immortalised cell lines, a potential challenge could be harvesting sufficient quantities of CTCs from patient samples for use in conjunction with whole blood samples bioimprints. Cell retention from complex whole blood suspension has significant impact on the selectivity. This is a key disadvantage when considering biosensor technology for CTC isolation. Capture of CTCs have shown promise in providing genotypic information of the primary cancer cell type. Abundance and type of CTC can indicate location and progression of a primary tumour. Diagnostically, this is of little use in treating primary cancers as patient are likely to present with symptoms in order to be evaluated. However, CTC detection of tumours known to be associated with late presentation and poor outcome may improve prognosis of these cancers with particularly poor prognoses. Capture of CTCs by bioimprints is more promising in detecting metastasis as malignancy spreads to secondary locations in the body via the blood system. Monitoring CTC levels in blood samples would allow fabrication of an indicator of metastasis. A further promising use of CTC biosensors is in liquid tumours such as acute and chronic leukaemias. Lowering the limits of detection of minimal residual disease for leukaemia patients would provide clinicians an earlier warning the patient is entering relapse and thus improve prognosis by enabling the opportunity to give pre-emptive therapy before an overt clinical relapse.

Bioimprinting has also been used to achieve nanometre scale resolution imaging of cancer cells. Though an underdeveloped field, promising studies of the drug species effects on cancer cells have been trialled on cells *in-vitro*. In this fashion, it is possible to apply chemotherapeutic agents and analyse the effect on surface morphology which has long been known to be responsible for the function of the adhering cells. In this setting bioimprinting provides a promising route to understanding chemical triggers and signalling processes associated with developing cancers. The major benefit of such studies is being able to provide tailored therapy after characterisation of malignancies via their extracellular features.

Artificial imprinted recognition entities can also be key to safe and efficient drug delivery vehicles. Studies have shown the promise in using imprinted microparticles to target tumour cells, however reports so far are only *in-vitro*. Due to the complementary binding nature, chemotherapeutic agents can be applied directly to dividing cancer cells. Thus, significantly higher doses can be used to yield devastating effects solely on malignancy and ablate selectively cancerous tissue. Ultimately, specific drug delivery vehicles make treatment safer and more accessible to all patients.

Acknowledgements

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ORCID

Jevan Medlock: 0000-0003-2425-3777
Anupam Das: 0000-0003-1948-8811
Leigh A. Madden: 0000-0002-1503-1147
David Allsup: 0000-0001-6159-6109
Vesselin N. Paunov: 0000-0001-6878-1681

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