Development of Nanoballoons for detection, isolation and analysis of disseminated disease in breast cancer.

National Breast Cancer Foundation

Ingham Institute Applied Medical Research

OBJECTIVE: To use nanobots for cell-based diagnosis, and as drug delivery vehicles in cancer. To do this we will:

- Develop (antibody & aptamer) conjugated ***bio***compatible metal-organic framework (MOF) nanobots for marker specific cell recognition and isolation (**Aim 1**).
- Use a computational model to characterise cell-nanoparticle interactions, predict novel structural combinations and optimise nanobot formulation (**Aim 2**).
- Use optimised cell targeting nanobots for circulating tumour cell (CTC) isolation and investigation (**Aim 3**).

HYPOTHESIS: Autonomous nanoparticles (nanobots) can be used to target disseminated disease (CTCs) in cancer.

THE PROBLEM: Early detection of disseminated disease is the key to effective cancer treatment. Current imaging methods lack resolution needed to detect early disease (<2mm) [2,3], while molecular methods lack the cellular context needed to ascertain treatment course [4]. In addition, current CTC isolation protocols lead to changes in cell phenotype, which makes data from further *ex vivo* analysis problematic. Finally, anticancer drugs are often cyto-toxic and come with unwanted side effects, which makes application to all patients who might benefit problematic. The use of nanobot CTC isolation has the advantage of not relying on fixation protocols (cells stay viable), and unlike magnetic beads, nanobots can be easily removed for post capture analysis of the cells (**Fig. 1A**). In addition, because of their autonomous motion, neutrally charged nanobots have the potential to be used for efficient drug delivery in patients to target disseminated disease (**Fig 1B**).

SIGNIFICANCE: In breast cancer the development of resistance to endocrine therapy, associated with loss of hormone receptors, is a key reason for patient mortality [5]. However, despite the cellular heterogeneity observed in breast tumours, no single cell-based assay has yet been developed that can identify changes in cancer cell receptor status in the blood of cancer patients [6].

JUSTIIFCATION:

- Nanobots are effective at isolating specific cell types from a mixed cell population (**Fig. 2**).
- Nanobots can be dissolved in EDTA following isolation, leaving viable cells that can be investigated for malignant potential and determining patient specific drug treatment (**Fig. 3**).
- Nanobots can be used for the targeted delivery of cytotoxic anticancer drugs (**Fig. 4**).

NOVELTY: Nanobots for targeting of CTCs is highly novel. The use of specific aptamers (rather than antibodies) in the field of CTC research has the potential to significantly advance the field.

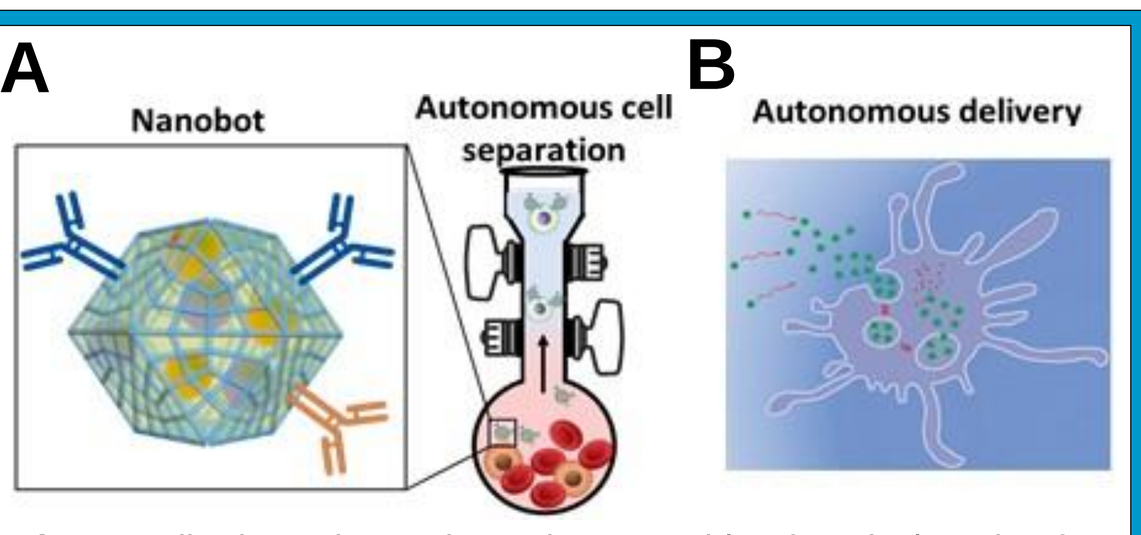

Fig. 1 Antibody conjugated nanobots capable of vertical motion for cell recovery [‘*find & fetch*’] (***A***), & cell targeting (***B***).

Approach. There are currently four major platforms used for CTC isolation: (i) Density Gradient Centrifugation (DGC); (ii) Micro filters/membranes; (iii) Immunomagnetic separation (e.g. CellSearch™); and (iv) Microfluidic chips [28]. These platforms are used separately or in conjunction with one another, and each method has its own set of limitations and advantages. DGC on its own is non-specific (enriches all mononuclear cells), results in inadequate separation purity and lacks sensitivity. Filters will clog easily, are limited by small processing volumes, and low separation purity: they will capture large debris or cell clusters. Immunomagnetic separation makes it difficult to retrieve captured cells as magnetic particles are permanently bound to CTCs, and there is low throughput as the process is time consuming; relying on trained pathologists to examine cells on slides. Microfluidic chips separate cells, however, it is difficult to retrieve captured cells as they are often permanently bound to substrate. While chips also suffer from problems of volume limitations and low throughput. In contrast to the older generation of CTC isolation methods, the use of nanobots has the potential to address all limitations in current techniques in one: (i) improve capture efficiency, purity, throughput, and release efficiency; (ii) lead to the analysis of almost unlimited sample volumes; (iii) be developed to be highly automated; and (iv) as nanobots are dissolved in EDTA, this will allow for the complete recovery of captured cells. As the topic of a recent Nobel Prize in Chemistry (2016), self-propelled nanomachines represent a new paradigm in nanobiotechnology. These autonomous devices gather energy from their surroundings, navigate, and can swarm to selectively search for specific cells or chemical species. Due to their unique advantages, synthetic nanomotors hold great promise for biomedical applications, such as targeted drug delivery, cell identification, biochemical sensing, nano surgery and bioimaging. Their use as bots for cell-based isolation has not been reported and seeks to fulfill a need not currently present in current CTC diagnostic methods

Preliminary Data. *Nanobot development.* We have demonstrated a simple fabrication method for these nanoparticles, which allows the precise control of size, morphology, and functionality in a single-step process. Nanobot are fabricated by encapsulating Catalase in porous MOF matrices. In the presence of hydrogen peroxide (H_2O_2, 50-200µM), nanobubbles are produced by a biocatalytic reaction ($H_2O_2 \rightarrow O_2$). Bubbles made this way are retained by the MOF, allowing it to shift its buoyancy; and driving it vertically in a liquid column (**Fig. 2**).

In **Fig. 2** we show that nanobots conjugated with mouse anti-human epidermal growth factor receptor 2 (Her2) antibody can be used to effectively separate $Her2^+$ BT-474 cells from a mixed population, also containing $Her2^-$ MCF-7 cells [ratio 1:50; BT-474:MCF7]. After mixing with anti-Her2 conjugated nanobots and introduction of H_2O_2 (100 µM), targeted cells are carried to the top of the liquid column (**Fig. 2**). Importantly, for the first time we showed that the nanobots can be completely removed by EDTA and the recovered cells regain their full metabolic and proliferation potential (**Fig. 3**). We also demonstrated separation of CSPG4 positive cancer cells from a mixed population using in house monoclonal antibody conjugated to nanobots (**Fig. 4**).

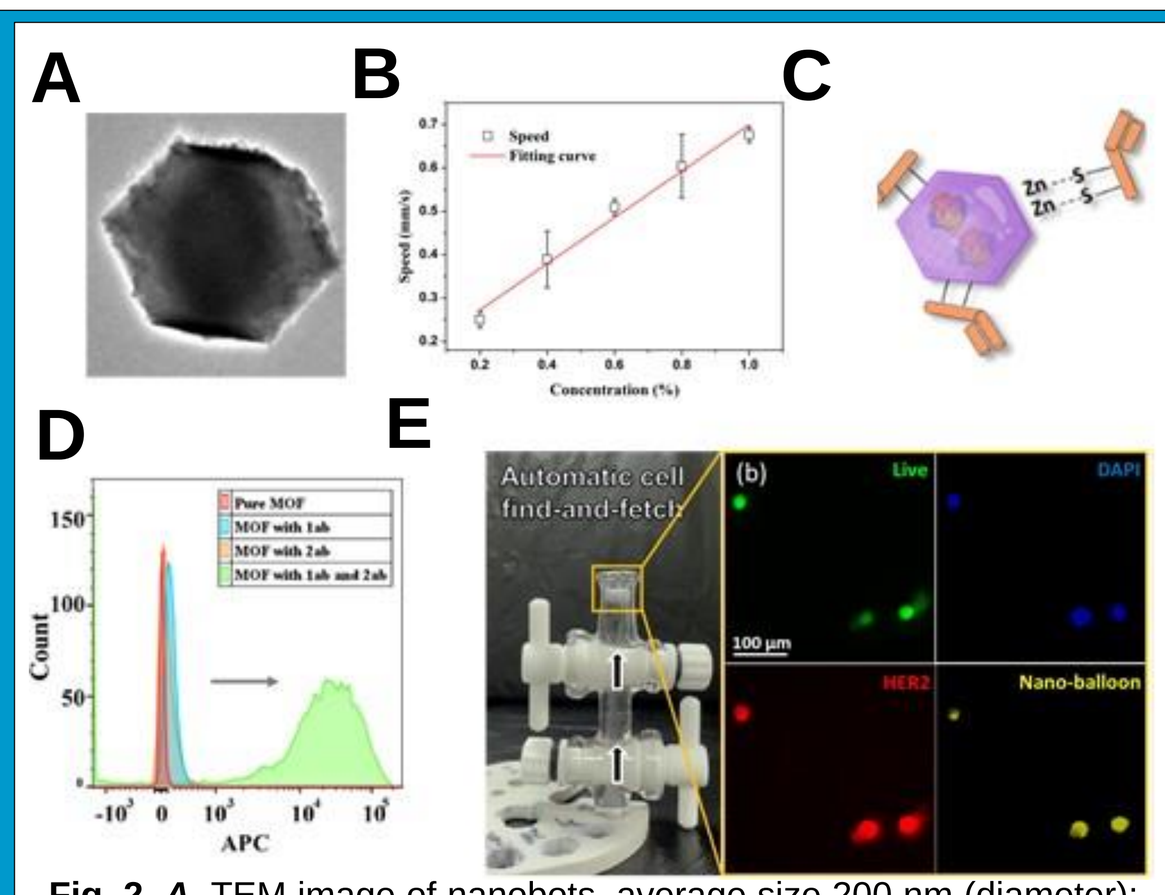

Fig. 2. ***A,*** TEM image of nanobots, average size 200 nm (diameter); ***B,*** Moving speed vs. in H_2O_2 concentration; ***C***, schematic of antibody conjugation through Zn-S bond; ***D,*** flow data showing the successful HER2 antibody conjugation; ***E,*** Prior to separation, nanobots and cell suspension are added to the bottom of the device (shown left). The mix is then aerated with H_2O_2. Experiments are performed at 4° C to minimise active cell internalisation of nano-balloons. Also shown, fluorescence microscopy of mixed BT-474 and MCF-7 cells after Her2-nanobots targeting and separation.

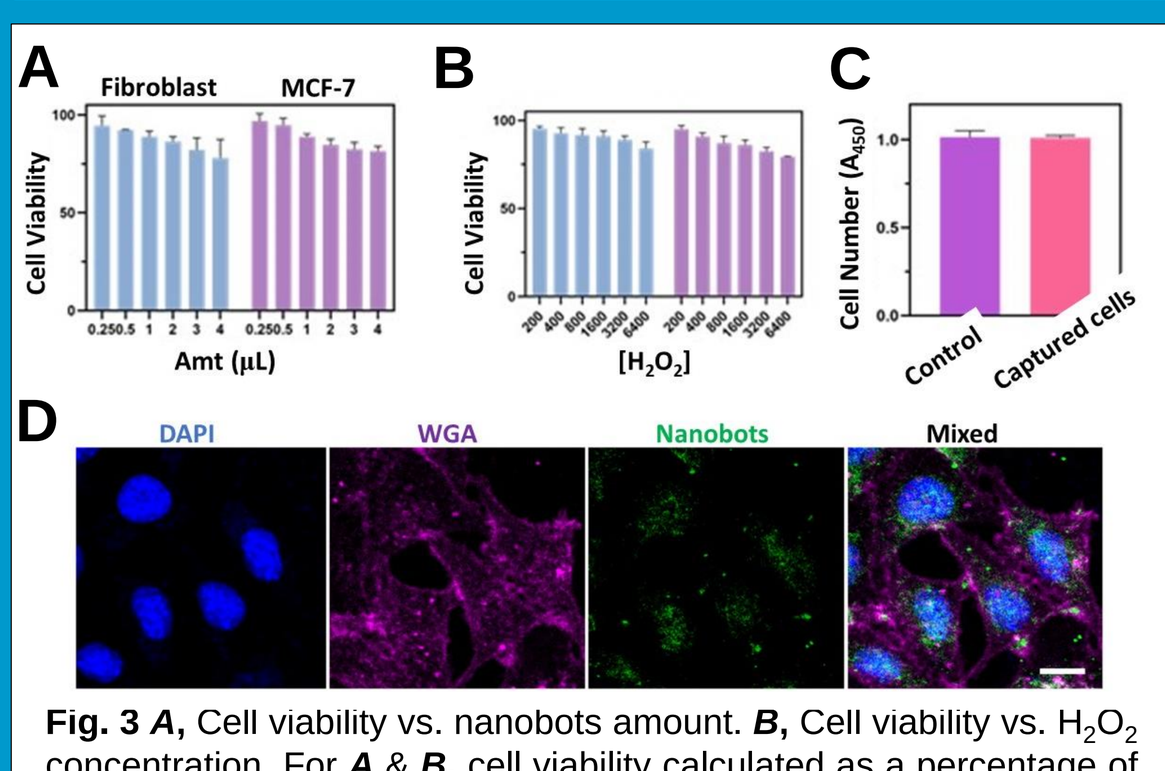

Fig. 3 ***A*****,** Cell viability vs. nanobots amount. ***B*****,** Cell viability vs. H_2O_2 concentration. For ***A*** & ***B,*** cell viability calculated as a percentage of total cells. ***C***, Cell proliferation potential after isolation, (viability Absorbance 450). ***D***, Fluorescent microscopy images of nanobot captured cells. Scale 10µM.

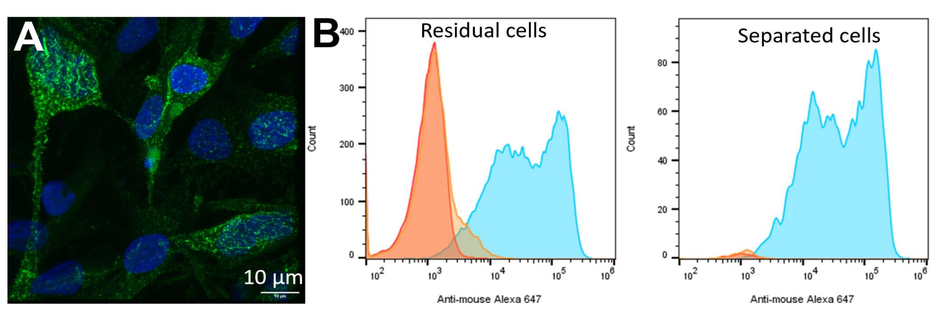

Fig. 4*A*, CLSM image of AF488-CSPG4 (green) antibody targeting MM200 cells (DAPI, blue). ***B*****,** FACS analysis showing the autonomous CSPG4-conjugated nanobot separation of MM200 cells (right) from mixed HUVEC and MM200 cells (left).

The focus of the grant will be to develop nanobots to identify and target two cell populations, that might be circulating in the blood of breast cancer patients, including $Her2^+$/$receptor^+$ cells as well as those that have lost receptor expression. In this last case, a marker linked to TNBC will be investigated (chondroitin sulphate proteoglycan/CSPG4).