Institut des Nanotechnologies de Lyon UMR 5270

Functionalized surfaces for the detection of circulating tumor cells (CTC) and bacteria

2- Surface functionalization strategies for CTC detection and analysis

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Challenges for CTC detection

☞ The discovery of CTC as a liquid biopsy had a revolutionary effect on early diagnosis, metastasis detection and personalized tumor treatment

☒ Use to detect the biological characteristic and molecular type of primary tumor cells
☒ Understanding their part in the metastasis may contribute to better therapeutic management

☞ Challenges:

☒ Rare: 1-100 CTC with 10^6-10^8 RBC /mL
☒ Heterogenous with variation in the expression of surface biomarkers
☒ Non destructive capture and detection methods for subsequent analysis : image analysis, cell culture, drug screening

☞ Selective capture of all CTC

☞ Release from surfaces without damaging the target cells

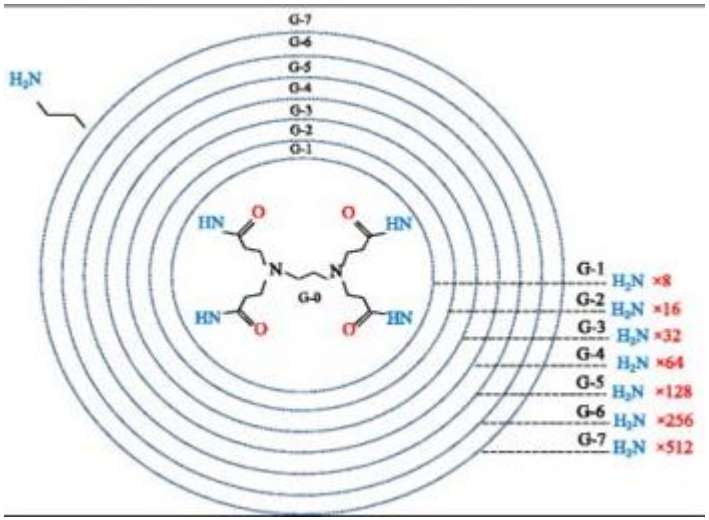
☞ Detection method for infinitesimally concentration in the blood stream

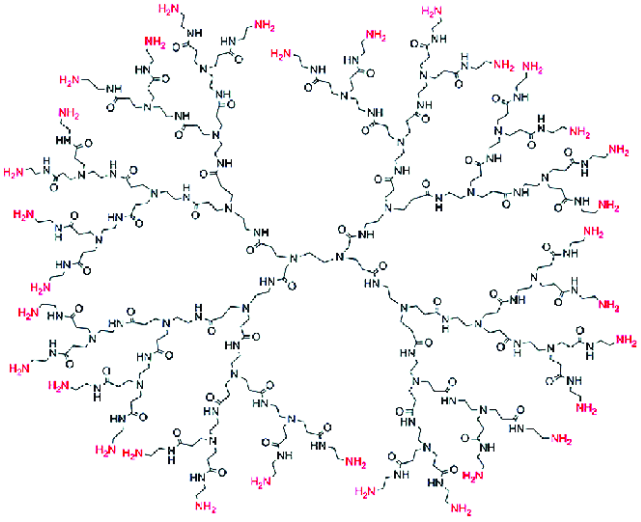
Non fouling surfaces

☞ One major problem for CTC detection device: non specific adsorption

☒ depends on hydrophilicity/hydrophobicity of the surface
optimal water contact angle for cell adhesion between 60° and 80°
☒ depends on charge of the surface
☒ depends on roughness of the surface

☞ grafting of dendrimers amino-terminated or carboxylated-terminated





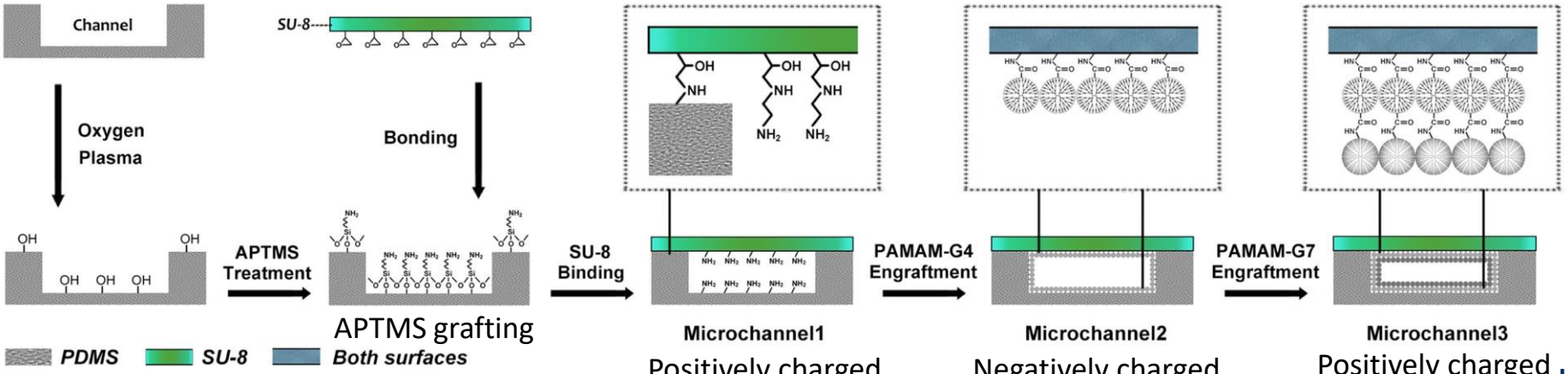
PANAM-NH_2 G3

150µm x 50µm

EDA grafting

PAMAM-COOH G4 grafting

PAMAM-NH_2 G7 grafting



APTMS grafting

Microchannel1

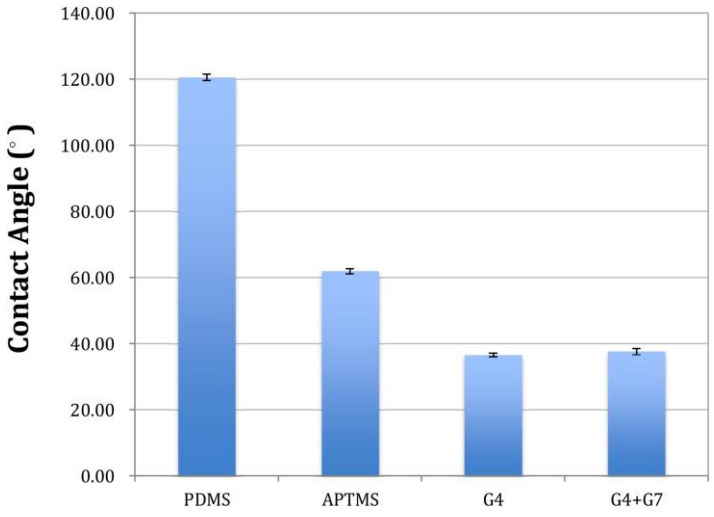
Positively charged

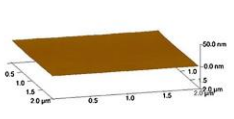
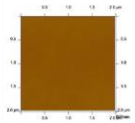
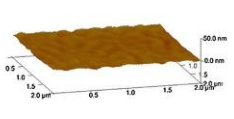
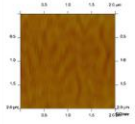
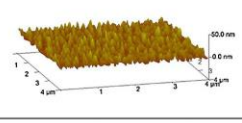
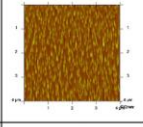
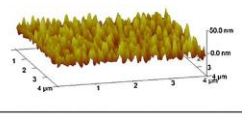
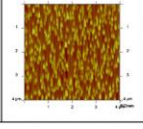
Microchannel2

Negatively charged

Microchannel3

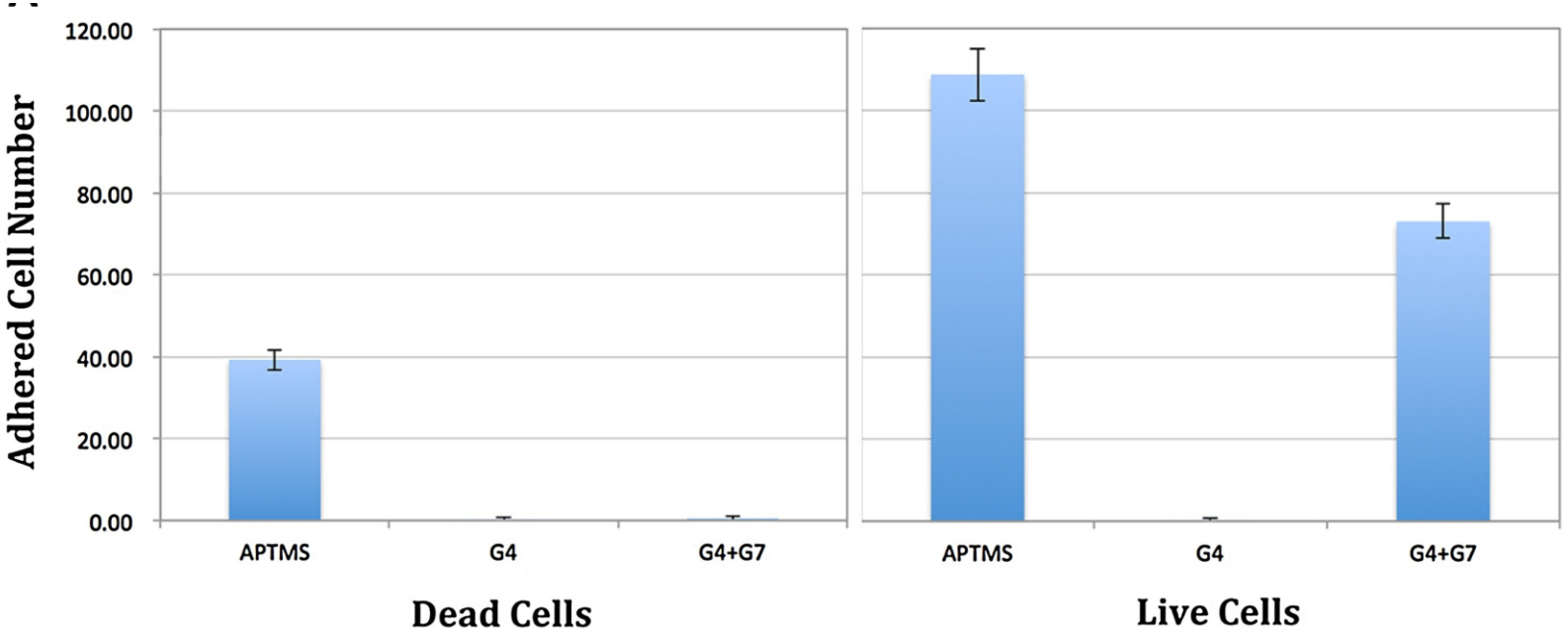
Positively charged



Groups	AFM 3D Image	Vertical View	Roughness
Unmodified PDMS			0.26±0.04
PDMS with APTMS			1.19±0.06
G4			3.62±0.23
G4+G7			5.88±0.33

Qin et al., Colloids and Surfaces B 2017

Injection of 10^6 CCRF-CEM cells/mL in PBS at 0.05 mL/h for 1h

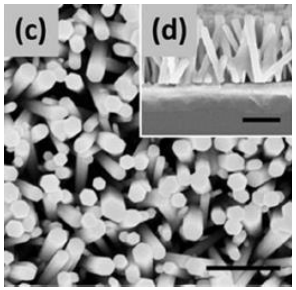


☒ Dead cells have no charge, Live cells are negatively charged

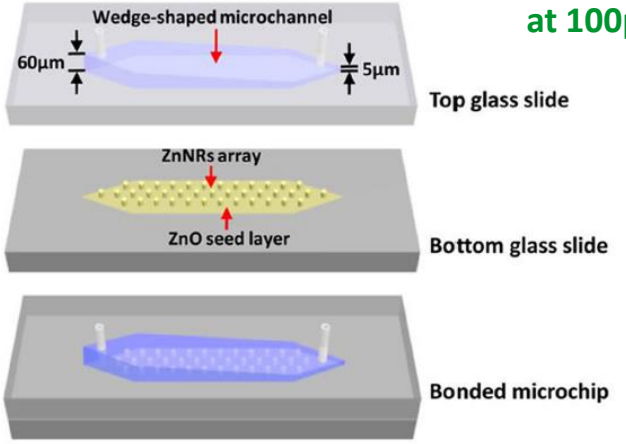
☞ Higher influence of charge surface than roughness or hydrophilicity (Tests done in PBS only)

Implementation of nanomaterials with microfluidic

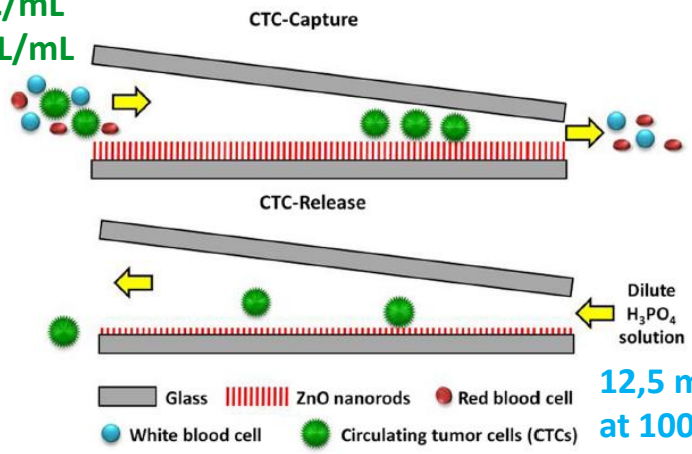
☞ Microchip with ZnO nanorods on glass slide to capture and release CTC



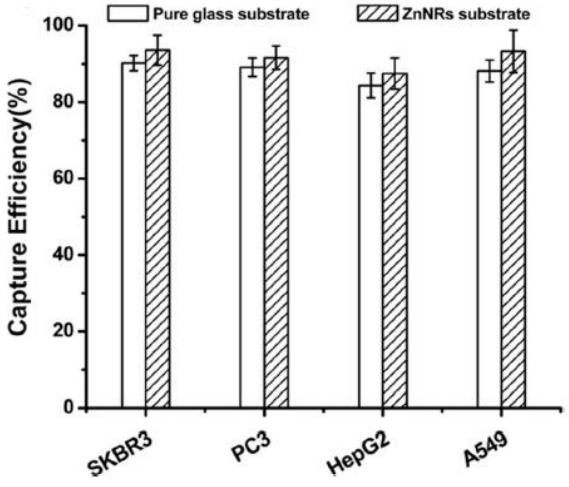
150-300nm in Dia
2µm in Length

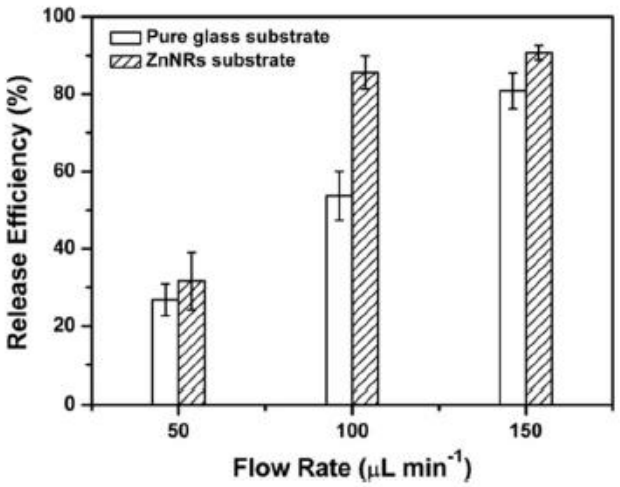


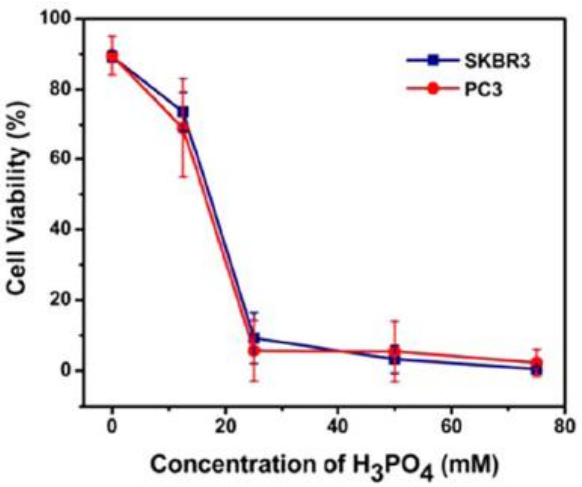
200 CTC/mL
at 100µL/mL



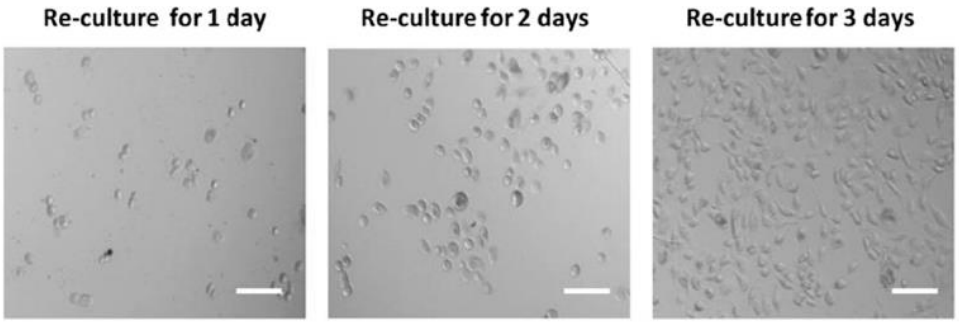
12,5 mM
at 100µL/mL







Li et al., Biomed Microdevices 2017



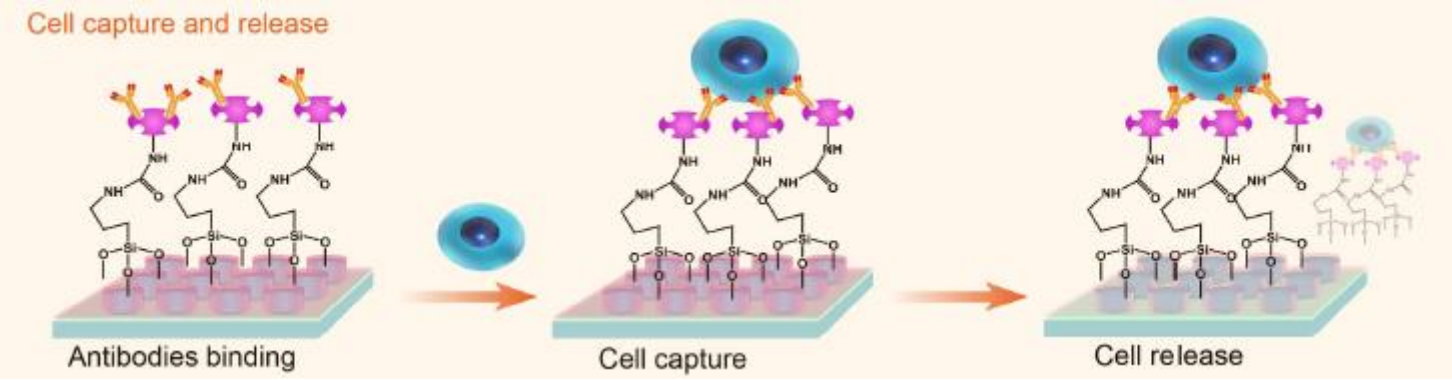
- CTC capture efficiency ≈ 85%
- CTC release efficiency ≈ 85%
- CTC viability at 1 day ≈ 70%
- CTC viability at 3 days ≈ 90%

☞What about CTC integrity?

☞ PDMS-based microfluidic chip with ZnO nanograss

80µm high
160µm Dia
260µm center-center pillar dist

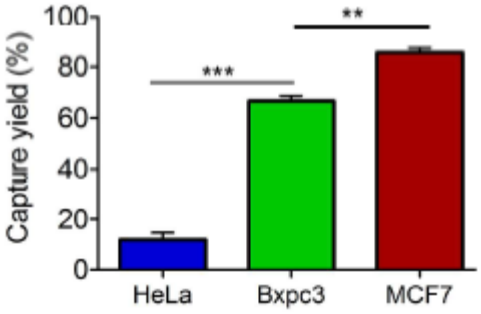
Hui et al., ACS Appl. Mater. Interfaces 2017



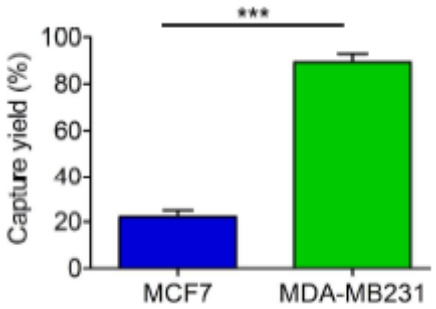
☒ ZnO nanograss reduce non specific adsorption of WBC

☞ EpCAM is epithelial cell biomarker; Vimentin is mesenchymal cell biomarker

1000 cells/mL blood



Anti-EpCAM microdevice



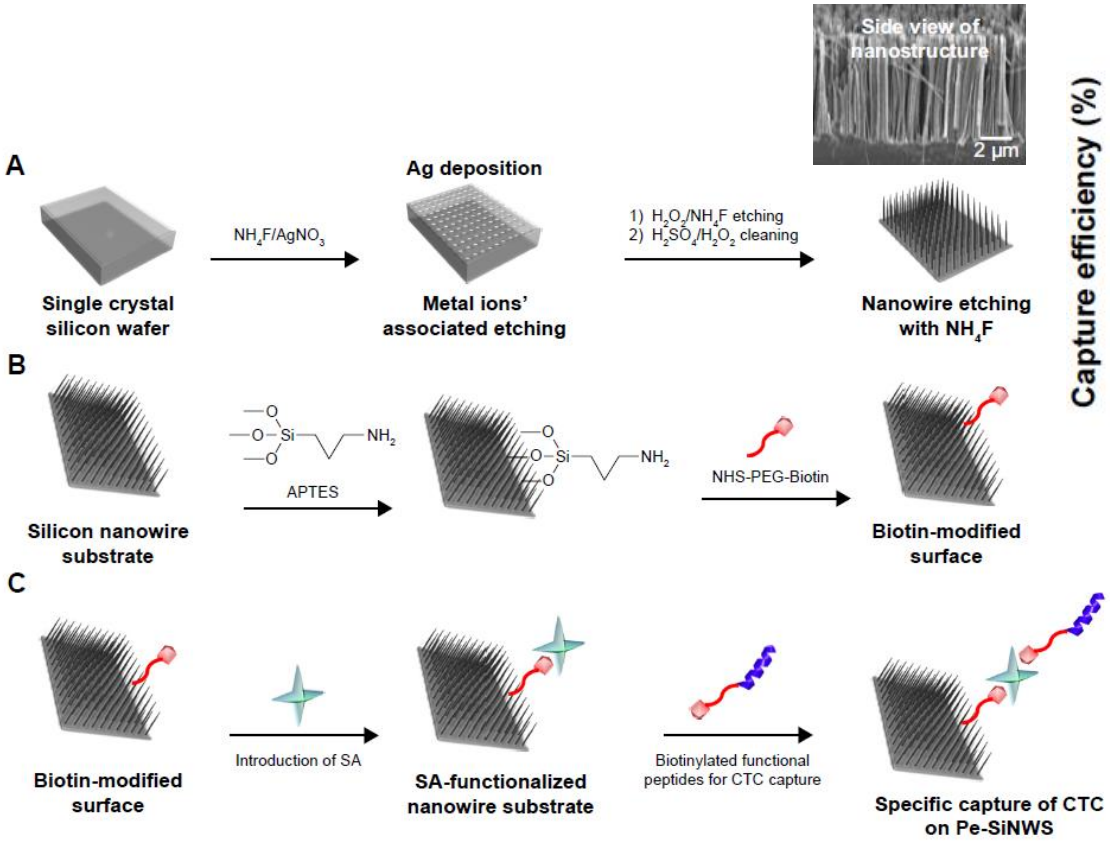
Anti-vimentin microdevice

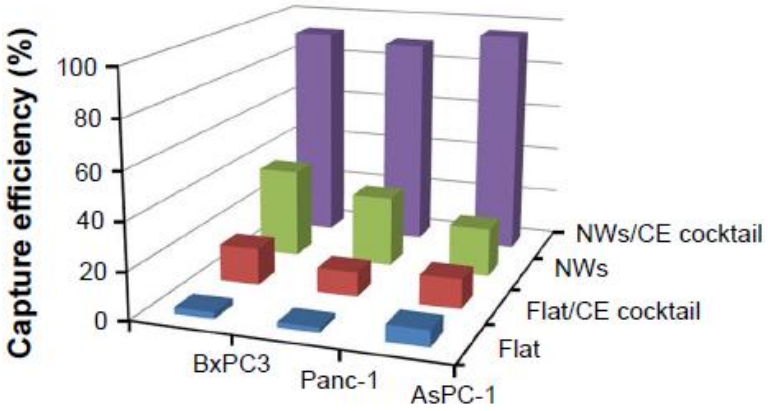
- Possibility to detach CTC from antibody functionalized ZnO nanograss by applying 0,1M HCl solution (pH5,6) with cell viability of 90%

☞ Improve pancreatic CTC capture efficiency by combining 2 specific interactions with microfluidic device

☒ EpCAM positive CTC with EpCAM recognition peptide (Pep)

☒ EpCAM negative CTC with CKAAKN peptide specific for pancreatic cancer cell

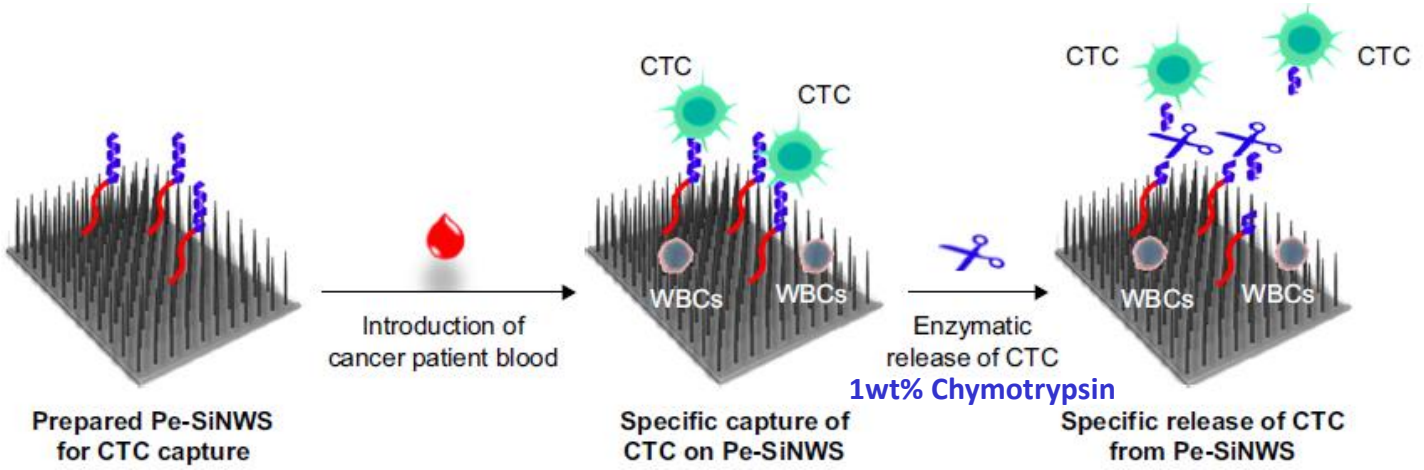


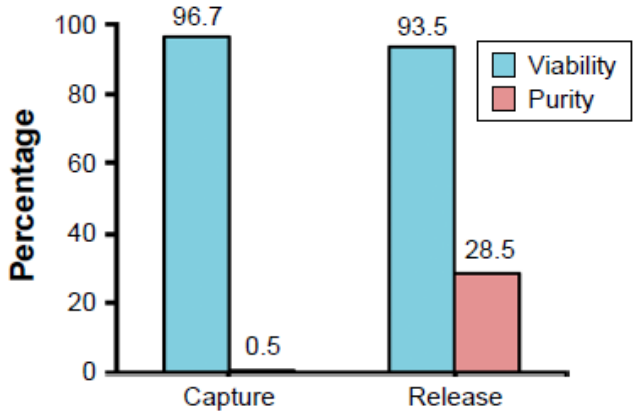


(200 CTC + 2.10^5 WBC) in 200µL

- High efficiency CTC capture with the cocktail peptides and the silicon nanowires design

Shen et al., International J. Nanomedecine 2019

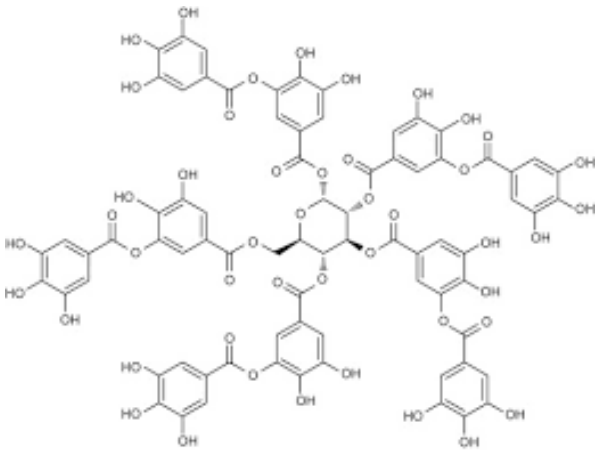




- Very good efficency of CTC capture and release
- Very good CTC viability
- But very bad purity rate due to WBC non specific adsorption onto Si nanowires!

Functionalization with chemical target of CTC membrane receptor

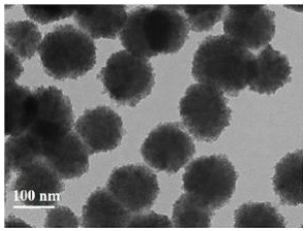
☞ Tannic acid on magnetic nanoparticles (MNP)

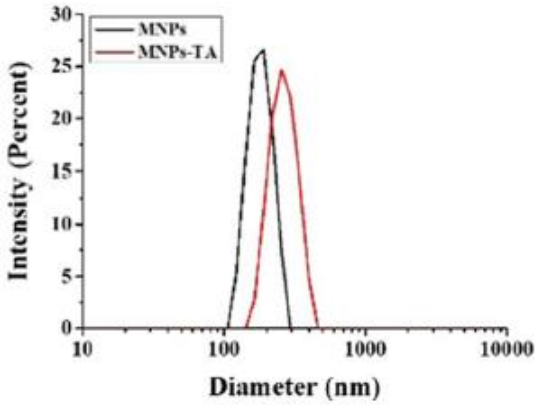


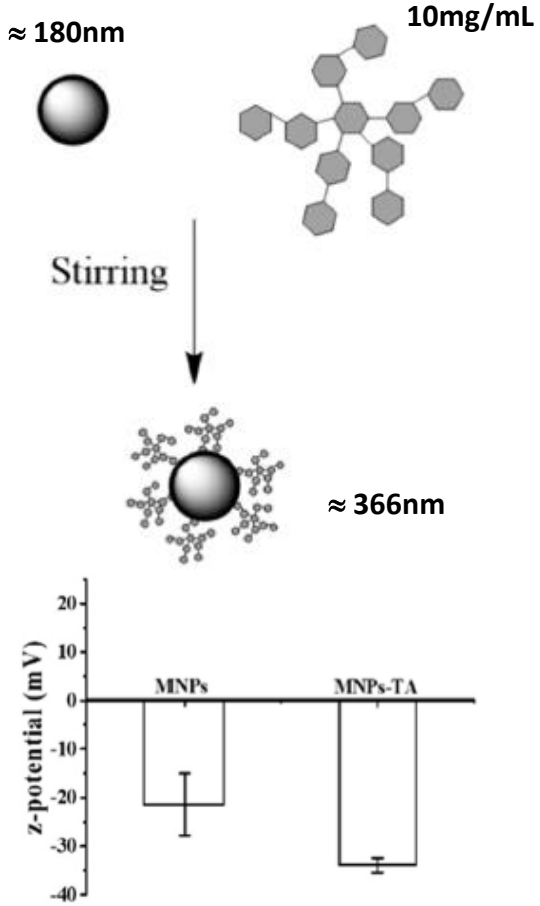
Tannic acid (TA)

- Interact with K residues of PKM2
- Anti-adhesion effect on WBC

MNP = $FeCl_3$ + PEG4000

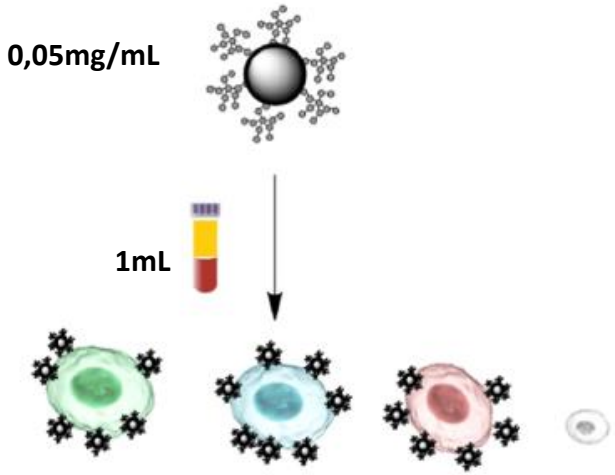


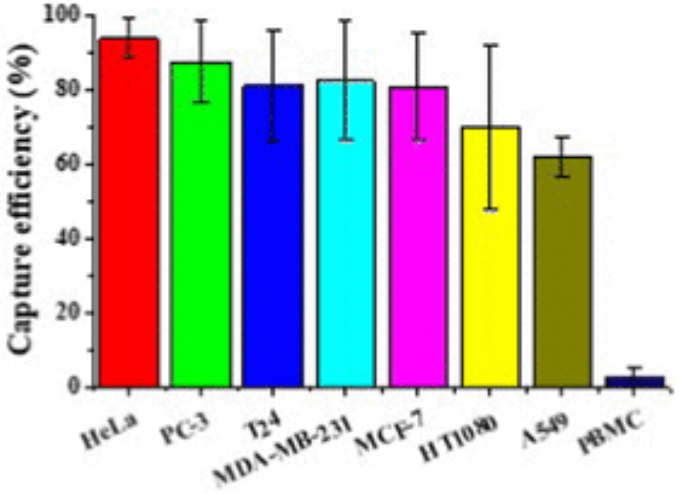




☒ FTIR analysis and UV-Vis spectra confirm the coupling of TA on Fe_2O_3 MNP

Ding et al., ASC Appl. Mater. Interfaces 2021





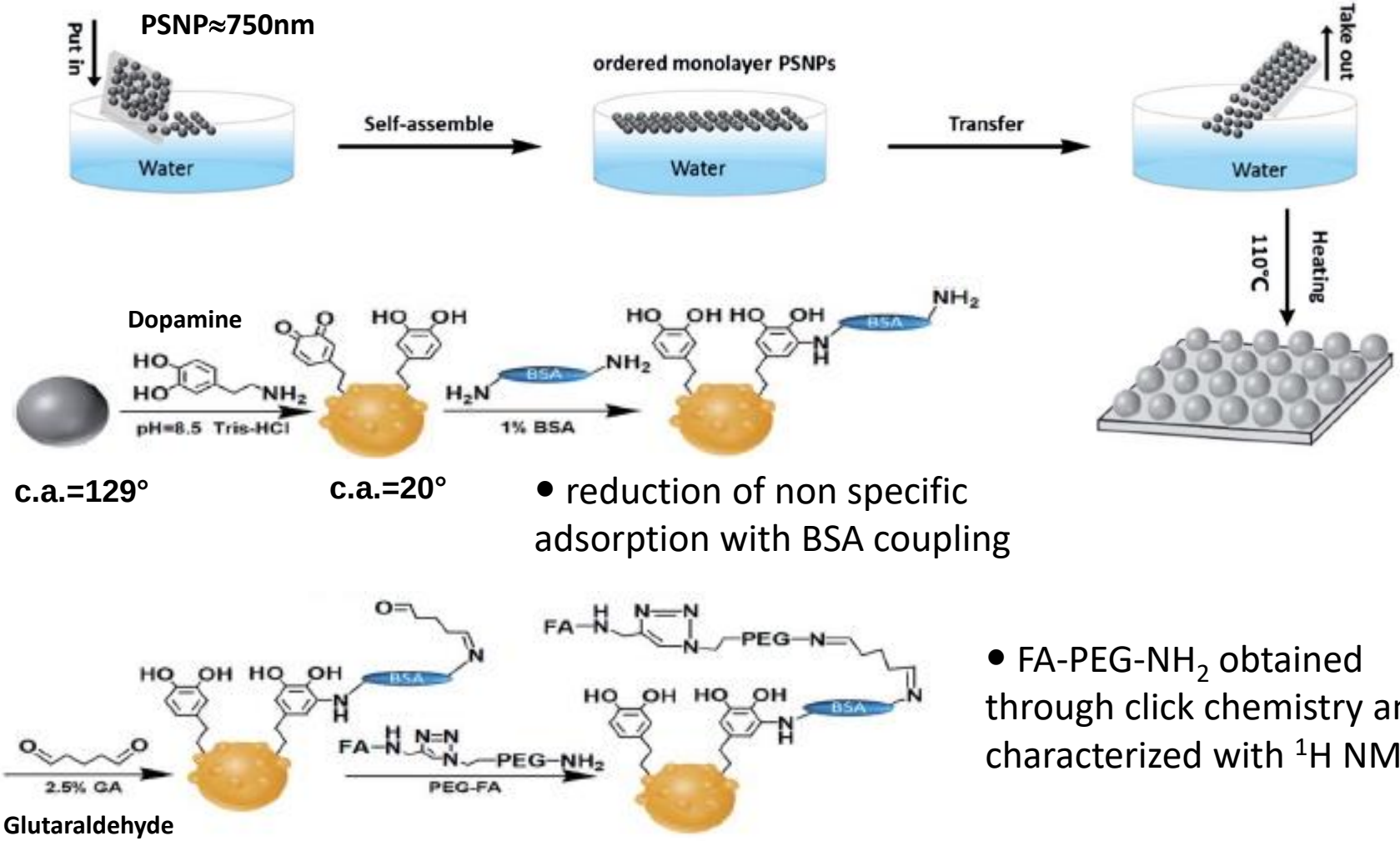
- 62-93% capture efficiency on 7 cancer cell lines

☞ CTC count in clinical samples:

☒ 21 cancer patients (8 type of cancers): from 1 to 10 CTC detected in 1mL blood

☒ 6 healthy donors: no CTC detected

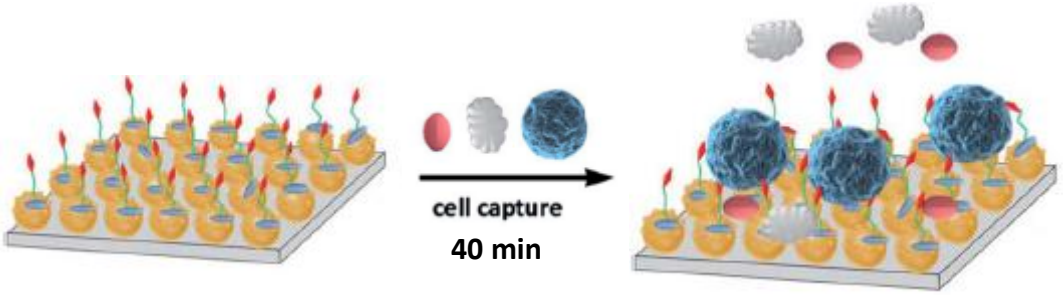
☞ Folic acid on polystyrene nanosphere (PSNP)



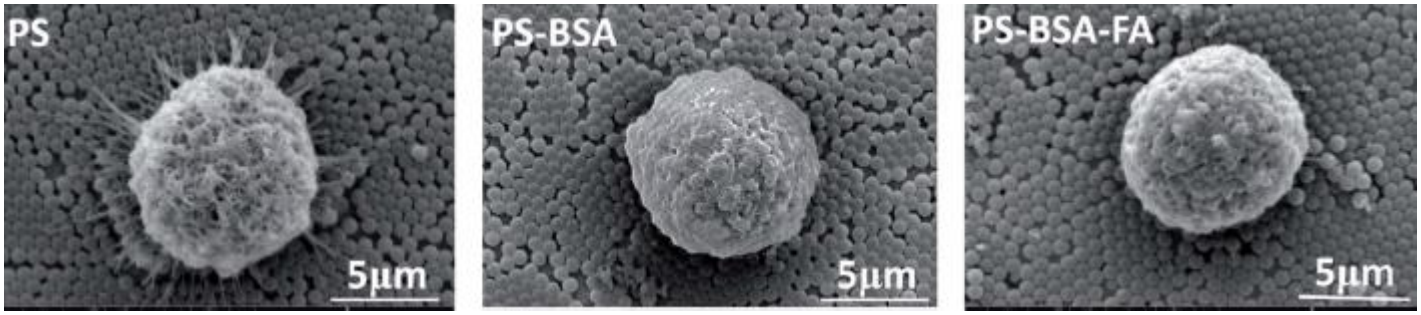
- reduction of non specific adsorption with BSA coupling

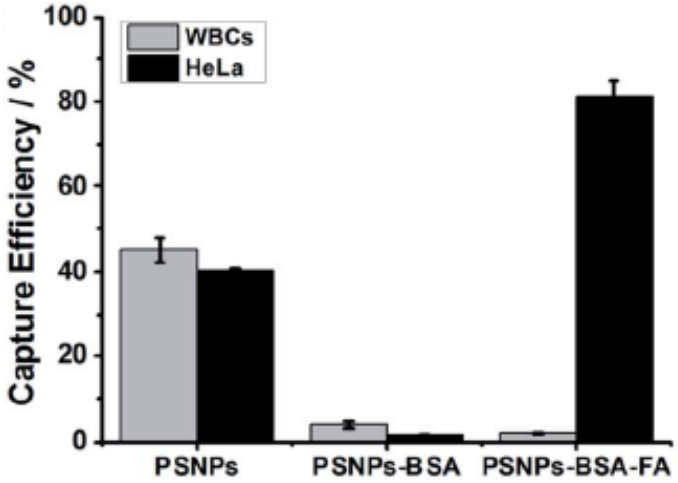
- FA-PEG-NH_2 obtained through click chemistry and characterized with ^1H NMR

Chen et al., Anal. Methods 2019



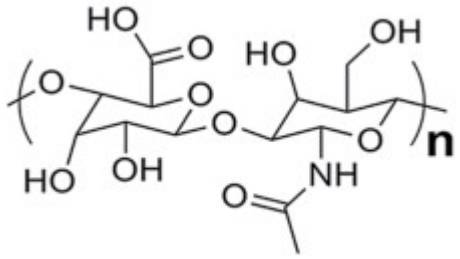
From 10 to 200 cell/mL





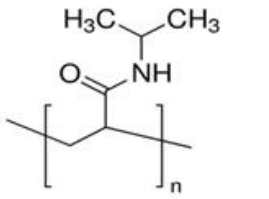
- FA-PSNP efficiently capture HeLa cell from (WBC + HeLa cell) sample
- Efficiency decrease to 40% in blood sample

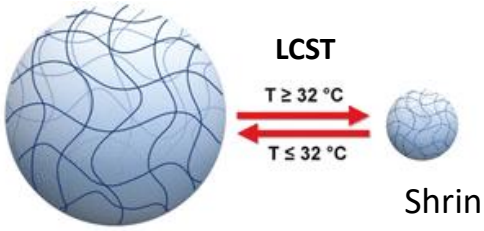
☞ Hyaluronic acid (HA)-based microgel



☒ HA is a glycan of ECM, known to promote tumor grouwth through interaction with CD44 cell receptor

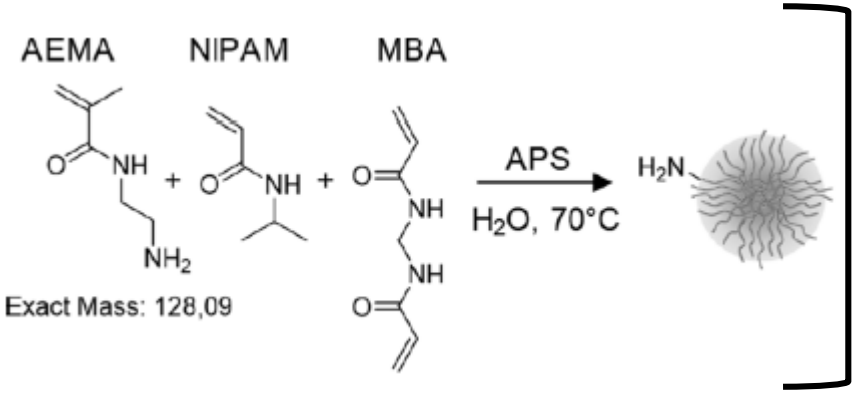
☒ PNIPAM (poly(N-isopropylacrylamide) is a thermoresponsive polymer

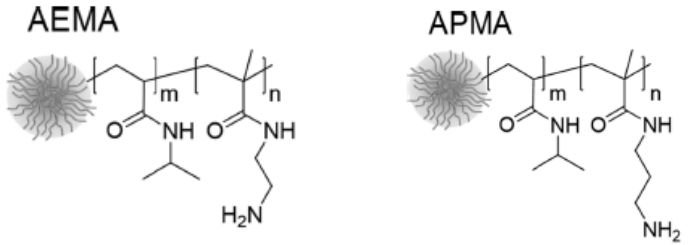




Swell (hydrophilic interactions)

Shrink (hydrophobic interactions)





Thermosensitive polymers

Schmidt et al., ASC Appl. Biio Mater. 2021

APMA

$R_h20 \approx 293nm$

Succinic anhydride (SA)

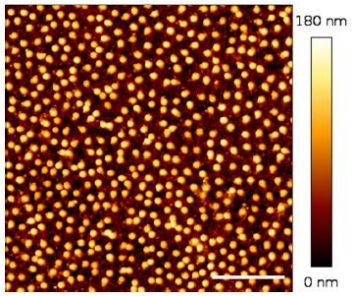
APMA-SA

$R_h20 \approx 356nm$

HA, EDC, NHS

APMA-HA

$R_h20 \approx 238nm$



AEMA-NPh-HA

$R_h20 \approx 735nm$

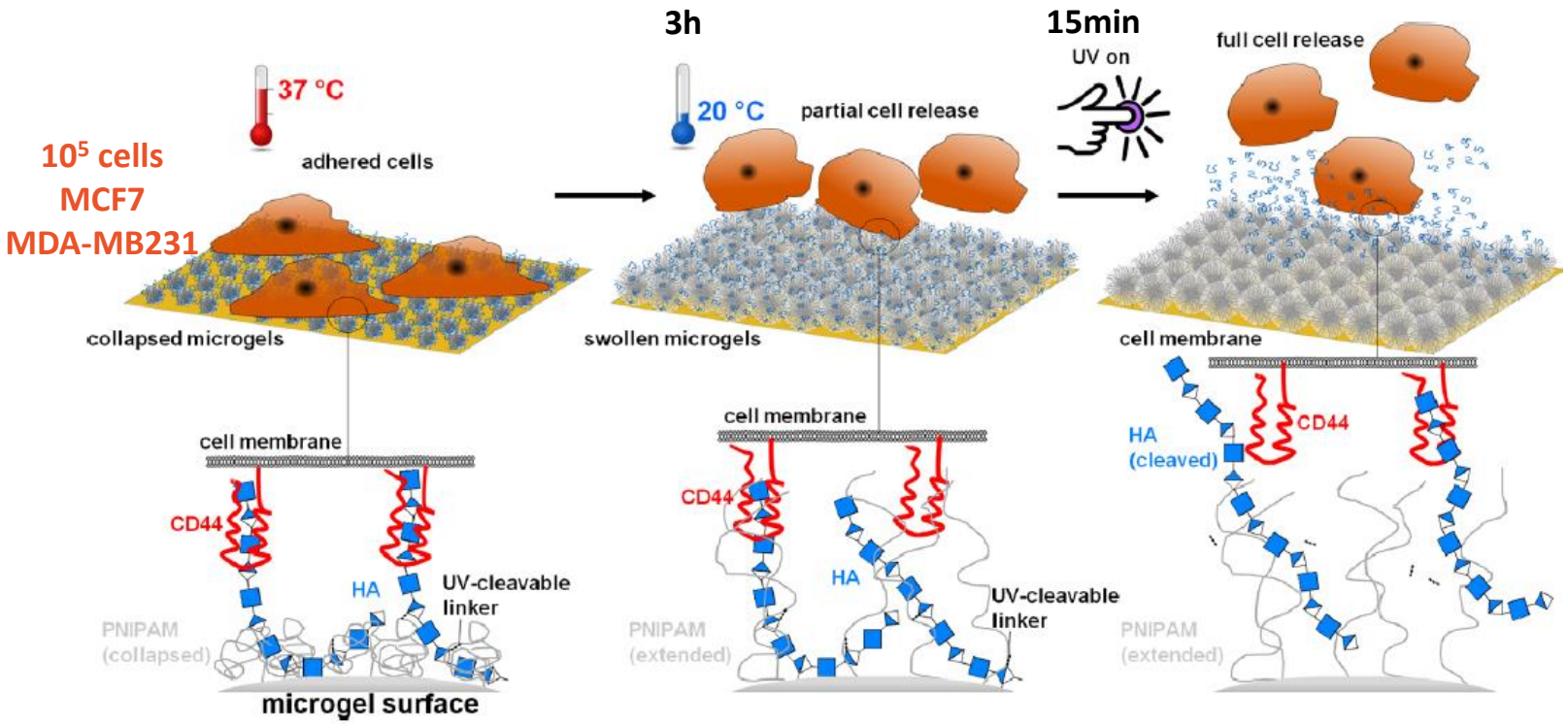
☒ Coating of microgels on hydrophobic silanized glass slide is homogenous and stable

AEMA

$R_h20 \approx 417nm$

NPh (photolabile)

HA, EDC, NHS



☒ Higher adhesion of MDA-MB-231 than MCF7 due to level of CD44 expression

☒ Reduced adhesion in the presence of serum proteins

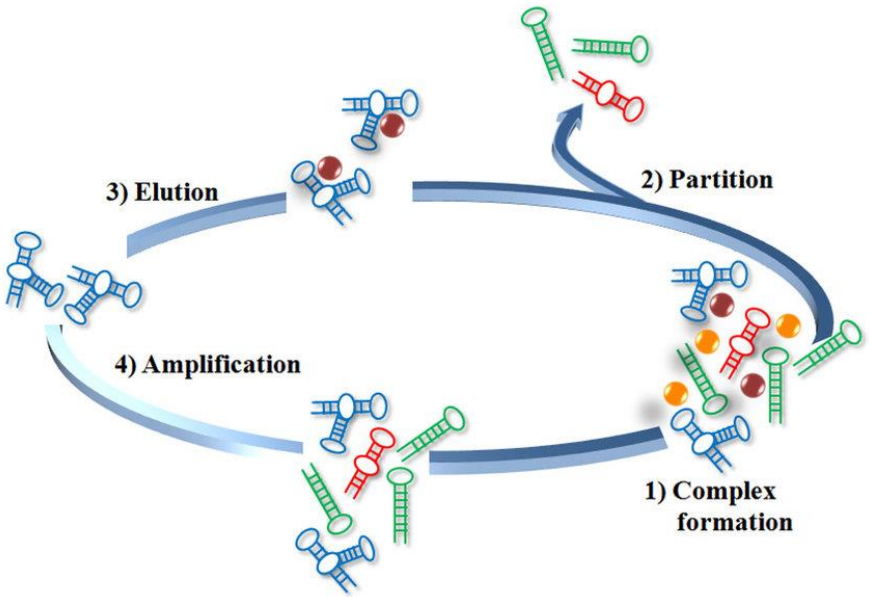
☒ Release up to 90% of adherent cells

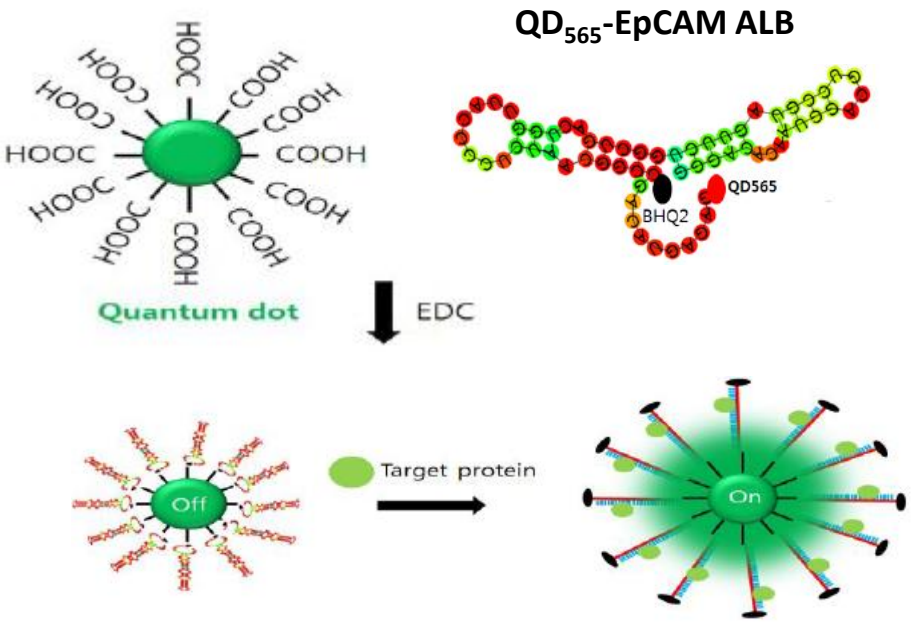
☒ Release of the remaining adherent cells

Aptamer-based CTC detection

☞ Aptamer = short single strand DNA or RNA (15-100nt) interacting with various targets

☞ selection through SELEX (Systemic Evolution of Ligands by Exponential enrichment)



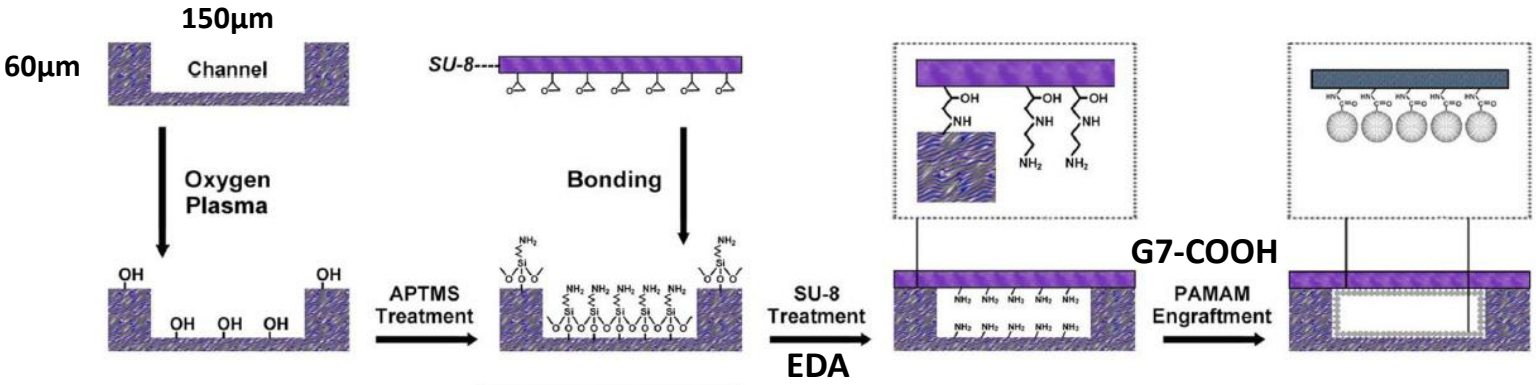


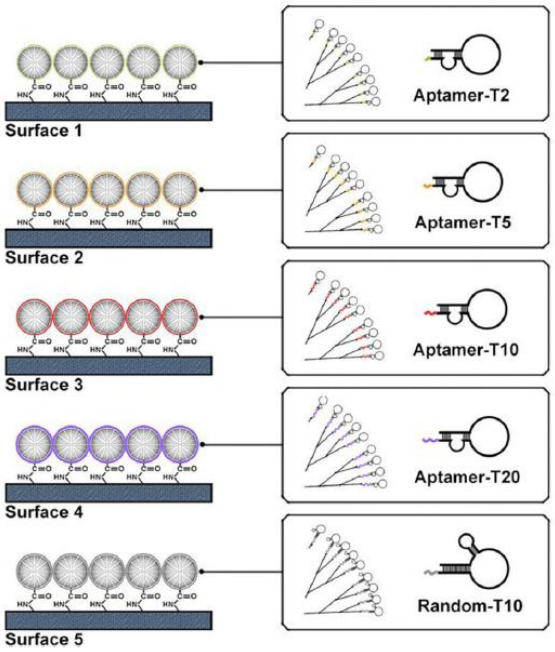
Hwang et al., Sensors 2016

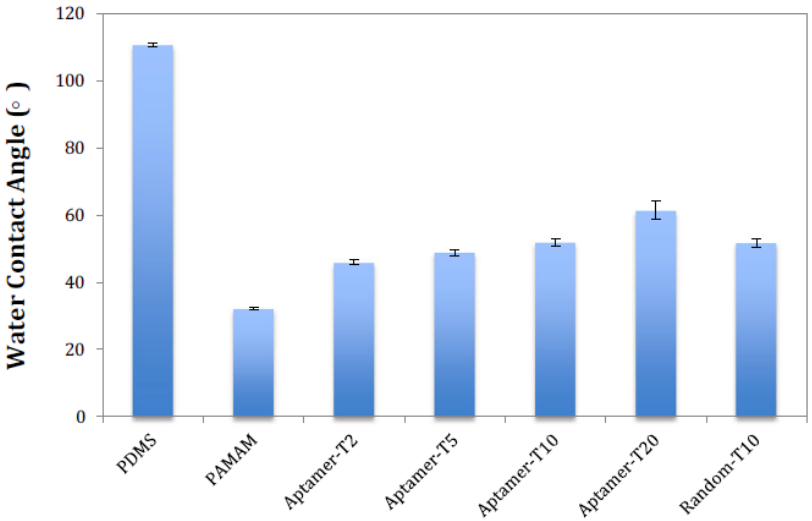
☞ Fluorescence intensity of CTC in a dose-dependent response with EpCAM expression

☞ Immobilization of aptamers into microfluidic channel

☒ Sgc8 aptamer specific for CCRF-CEM cells

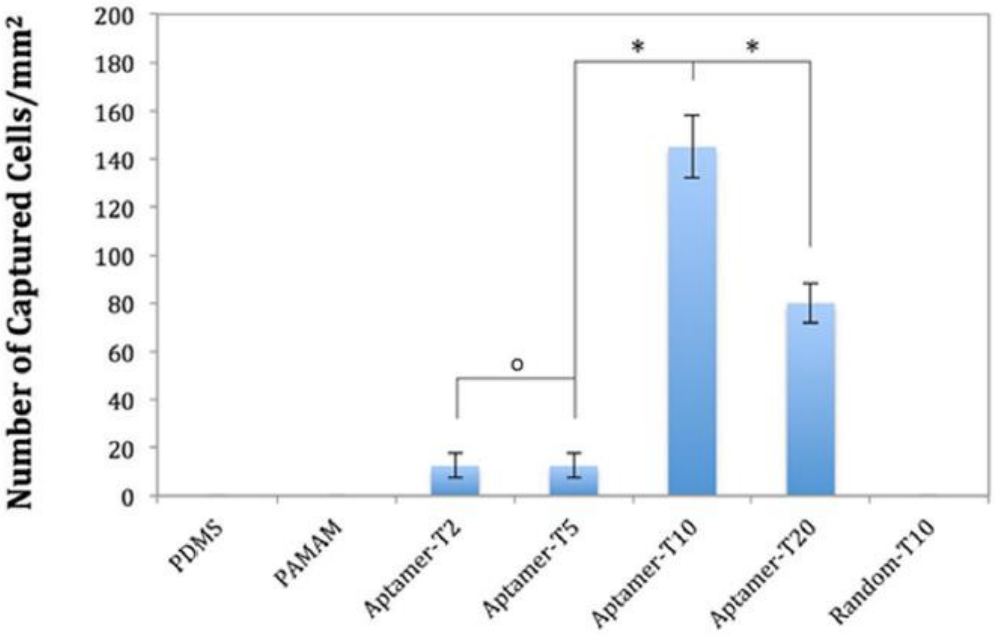






Qin et al., Biomed Microdevices 2019

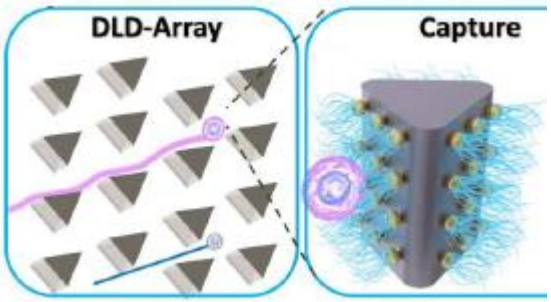
☒ Capturing performances of aptamer-functionalized surfaces by introducing 10^6 cells/mL at a flow rate of 0,05mL/h for 40min

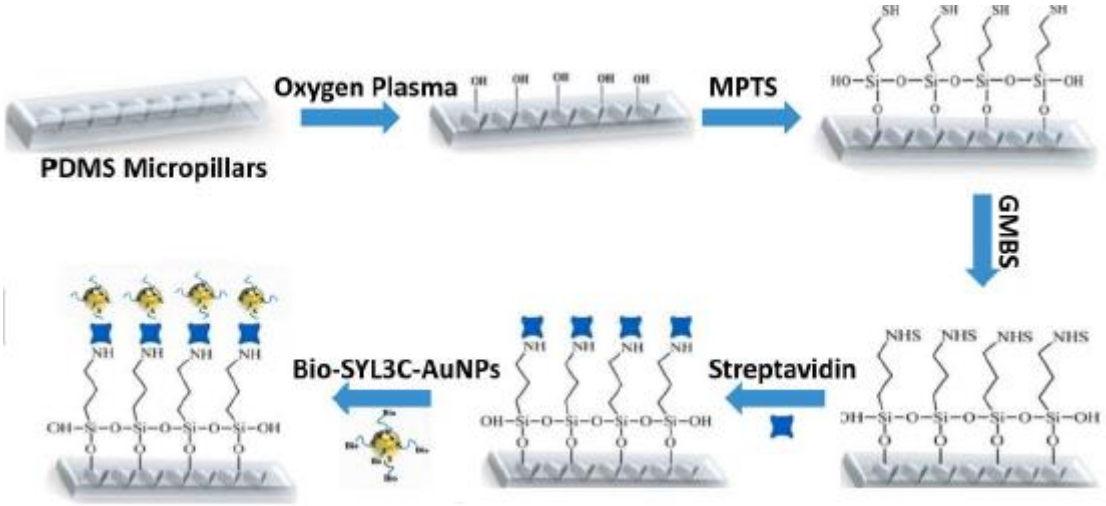


☞ Importance of the length of the spacer to improve cell capture and detection

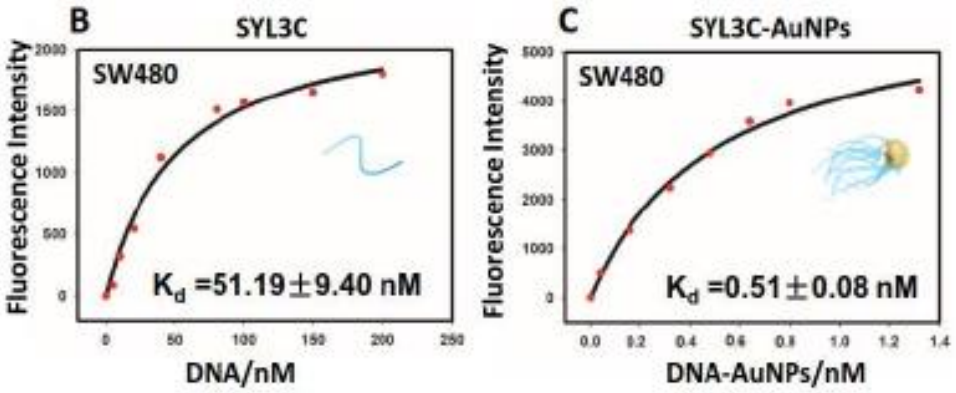
☞ Multivalent aptamer Octopus Chip

Aptamer SYLC3 specific for EpCAM immobilized on AuNP (16nm)



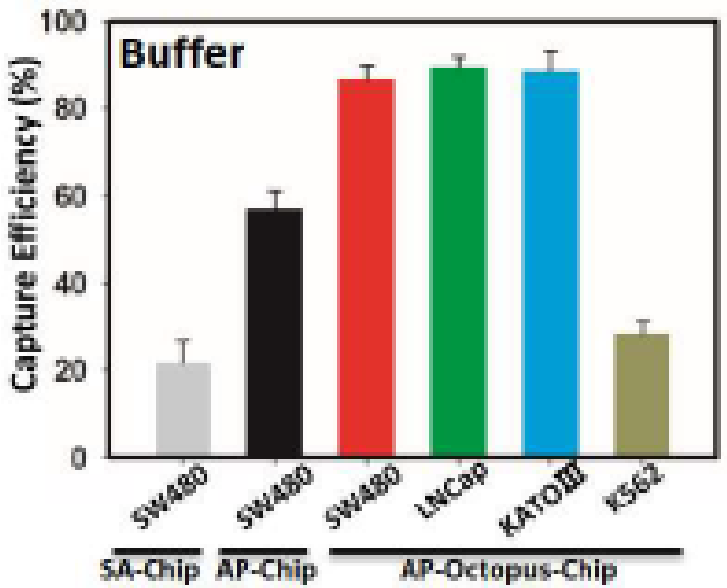


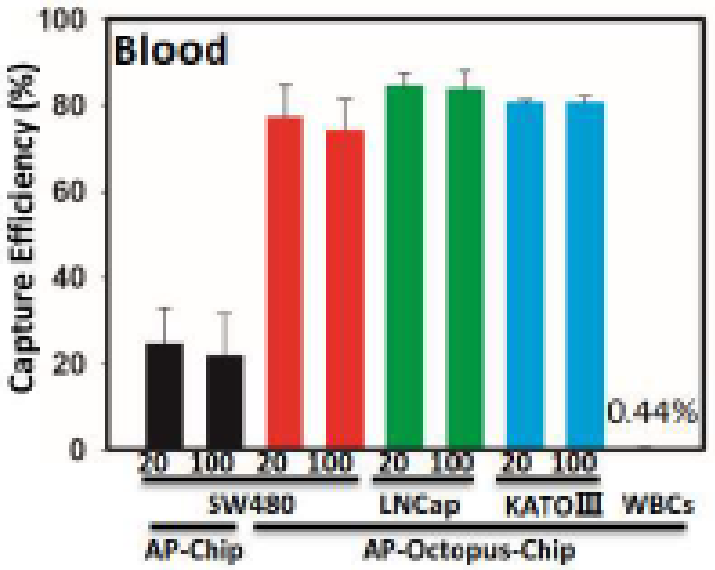
☒ Triangular micropillar increase contact time of CTC (>13µm) in the channel



☒ 100 times higher affinity with multivalent SYLC3-AuNP than with SYLC3 alone

Song et al., Angew. Chem. Inter. J. 2019

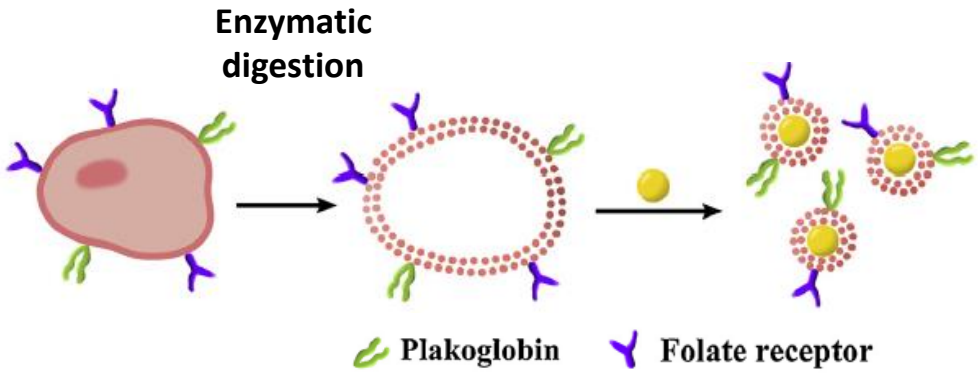




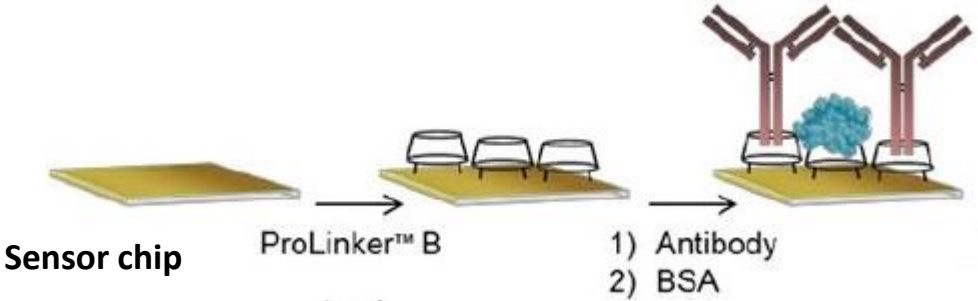
☒ Efficient and specific capture of 20-100 CTC in blood with 95% viability, and low non specific adsorption of WBC

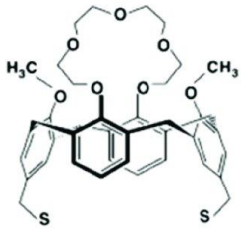
Membrane gold nanoparticles (M-AuNP) and SPR detection

☞ Enhancement of SPR signal with dual-selective CTC detection



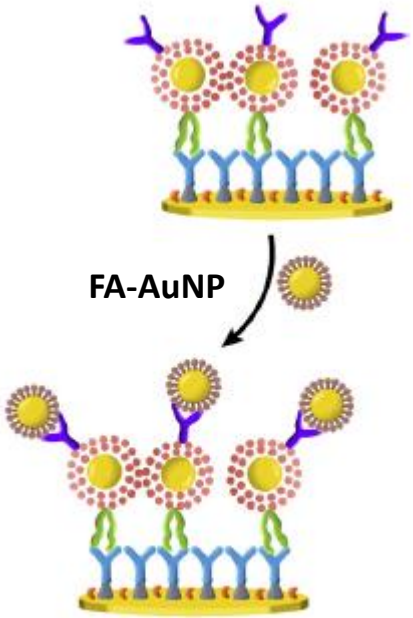
☒ Elaboration of M-AuNP
☒ Plakoglobin (JUP) is involved in desmosome junctions

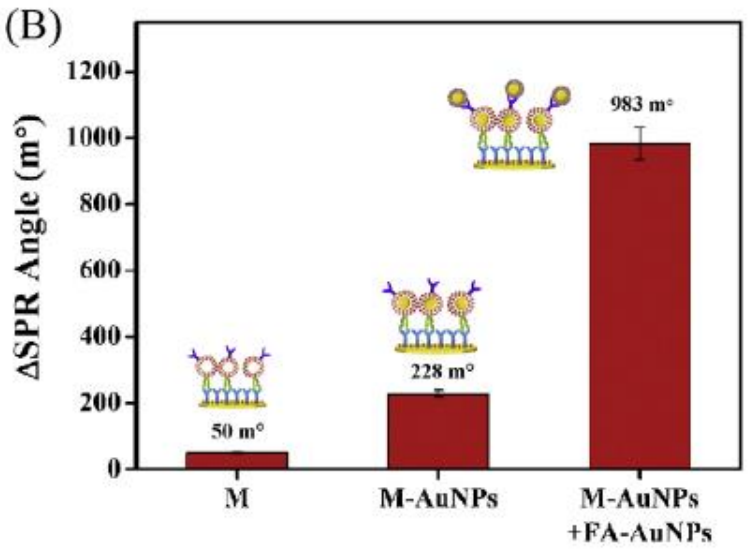


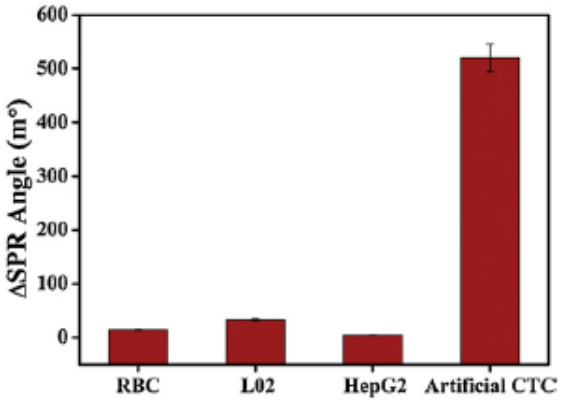


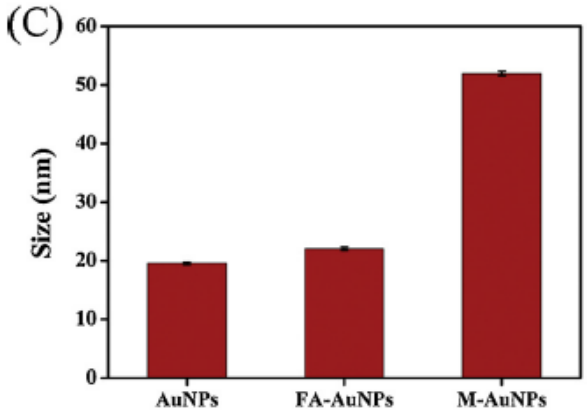
☒ Oriented immobilisation of the anti-JUP Ab through calixarene derivative interaction

Huang et al., Sensors and Actuators B 2020









☒ The combination of JUP/anti-JUP and FA receptor/FA with AuNP increase the specificity of the detection with amplification of the signal

☒ Detection limit = 1 CTC/mL

Conclusion

- Various methods for isolating, detaching and detecting CTC have been rapidly developed
  - ☒ need to increase specificity, sensitivity and throughput

- Effective release of CTC from the surface after capture without damaging the cells
  - ☒ enzymatic digestion
  - ☒ aptamer-mediated release
  - ☒ stimui-responsive polymers

- Many attractive advantages for microfluidic plateform
  - ☒ continuous sampe processing, with various functions (size, physical, electrical, marker-specific)
  - ☒ working at the cell dimension
  - ☒ But limitation due to possible clogging and low purity (physical-based), low input volume

- Need to establish and improve standardized protocols off CTC-related detection methods