

Research Project: Circulating Tumor Cell Isolation from Complex Matrices

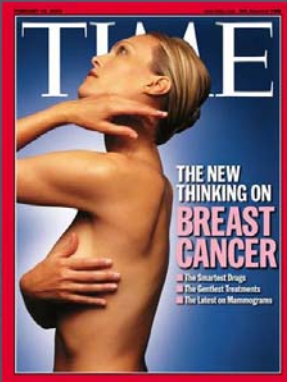
Introduction

Breast cancer represents the third most frequent cancer and encompasses nearly 9% of the total cancer burden worldwide. Breast cancer is characterized by development of adenocarcinomas in the ductal/lobular system of the milk ducts.

Breast Cancer

- ▶ > 211,000 cases in the US
- ▶ Detection primarily by mammography
- ▶ Misses ~10% of all tumors
- ▶ High rate of false positives
- ▶ Typical treatment
 - Excision of suspect tissue
 - Chemotherapy/Radiation
 - Continued monitoring

Singletary, S. E. and J. L. Connolly (2006). *CA Cancer J Clin* 56(1): 37-47.

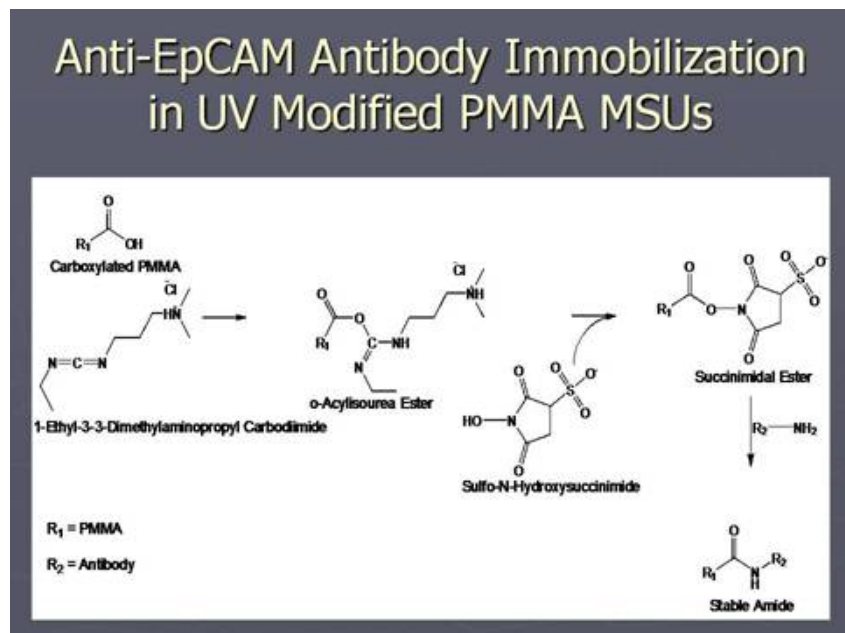


The tumors are prone to shed epithelial cells that reside in peripheral blood (1-10 tumor cells/ml in the presence of 10^7 normal cells/ml) and they are typically the sole epithelial cells found in peripheral blood [1]. The surface of the tumor cell is inundated with glycoproteins termed epithelial cell adhesion molecules (EpCAM) (3×10^5 molecules/cell) that have

three domains. EpCAM has an extracellular region, transmembrane region, and an intracellular anchor. A 5-mer (Leu-Phe-His-Ser-Lys) in the extracellular region of EpCAM has previously been demonstrated as an epitope for immunoaffinity based assays [2].

Theory

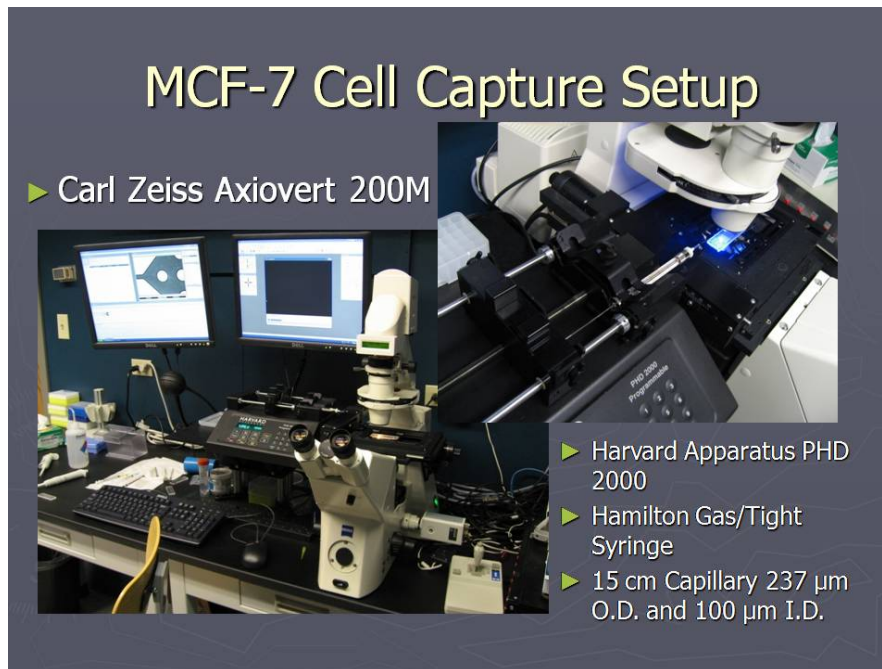
The foundation of this work has its roots in microfluidics; however, the mechanism that governs the process is immunoaffinity based. Specific combinations of hydrophilic, hydrophobic, van der Waal's forces, and hydrogen



bonding interactions conjoin to form stable interactions at the surface of antibody immobilized microfluidic devices and cancer cells. The degree to which the cells are bound to the surface was approximated using Bell's model [7].

Experimental

The volume capacities of microfluidic devices have typically been in the range of 0.25-0.50 μl making it difficult to sample low abundant targets [3, 4]. In order to sample large quantities of simulate, a robust high capacity microdevice was necessitated. A novel 17-channel microsampling unit with high aspect ratio microstructures (HARM) was fabricated using X-ray LIGA. Poly



(methylmethacrylate) (PMMA) was the substrate of choice for microsampling unit preparation due to the ease of surface carboxylation, demonstrated biocompatibility, and HARMs compatibility. The device consists of an intricate network of microstructures

with aspect ratios as high as 20:1 (See Figure 1). Exposure of PMMA to UV radiation at 254 nm with 15 mW/cm^2 power density for 30 min effectively produced a carboxylated polymer surface, which served as a functional scaffold to which anti-EpCAM antibodies were attached (See Figure 2) [5]. Effecting efficient cell capture in the microsampling unit required maximizing the probability of wall-particle interactions; accordingly, the channel cross-sections were designed with extreme rectangular character i.e. narrow (30-50 μm) and tall (250 μm) as to maximize such collisions.

Results and Discussion

The durability of the microsampling unit was demonstrated by passing blood simulate through the device with linear velocities from 3 $\mu\text{m}/\text{s}$ to 1.2 m/s . The endurance of the device was demonstrated by continuously pumping 250 ml aliquots of blood simulate through the device at 1 ml/min , and 10 ml aliquots of blood simulate were processed with the microfluidic device in less than 10 min. No device failures were observed due to clogging or leaking. The

immunoaffinity-based assays used to capture tumor cells from the peripheral blood simulatess were carried out using immobilized anti-EpCAM antibodies that were bound to the surface of carboxylated PMMA using 1-ethyl 3-dimethylaminopropyl carbodiimide as a surface activator [6]. Capture efficiency of less than 1.0% was observed when 10^5 cells were processed at linear velocities as high as 2 mm/s in 50 μ m width channels; however, when the channel width was reduced to 20 μ m the capture efficiency was ~100% (See Figure 3). In conjunction, these technologies have afforded us the ability to develop novel systems for capturing and processing low abundant cells from blood simulatess.

Corresponding Brightfield and Fluorescent Images of Captured CTCs

► Run Conditions

- Curvilinear 30 μ m wide 37MSU
- Axiovert 200M
- 40x

► Spiked Whole Blood

- 250 ± 20 MCF-7 cells/mL WB
- 1.0 mm/s L.V.

References

- [1] B. Simon et al., *PNAS*, 87(7) 2755-2759 (1990).
- [2] S. Szala et al., *PNAS*, 87(9) 3542-3546 (1990).
- [3] M. Galloway et al., *Electrophoresis*, 23(21) 3760-3768 (2002).
- [4] S. Ford et al., *Journal of Microcolumn Separations*, 10(5) 413-422 (1998).
- [5] N. Siampiringue et al., *European Polymer Journal*, 27(7) 633-641 (1991).
- [6] B. Oh et al., *Biosensors & Bioelectronics*, 18(5-6) 605-611 (2003).
- [7] Bell, G.I., *Science*, 200(4342) 618-27 (1978).