Multiplexed Fluorescent Assay of Testicular Germ Cell Tumors using a Centrifugal Microfluidic Device

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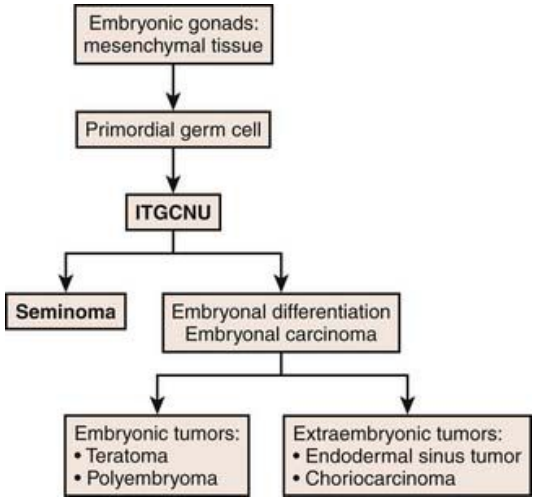
Background

Testicular cancer

- Gonadal germ cell tumors (GCTs) most common
- Diagnosed using ultrasound, blood tests, biopsy, CT, MRI, and PET

Multiple types of testicular germ cell tumors

- Seminomas
- Yolk sac tumors
- Choriocarcinomas
- Embryonal cell carcinomas
- Teratomas



Biomarkers

Staging and diagnosis of type of testicular cancer dependent on clinical examination, histopathology, radiology exams, and **serum blood markers**

- Allow differentiation between types of germ cell tumor
- Categories of markers:
  - α-fetoprotein (AFP)
  - β-human chorionic gonadotropin (β-hCG)
  - Lactate dehydrogenase (LDH)
- Useful in diagnosis, staging, in monitoring therapeutic response, and in detecting tumor recurrence

	AFP	β-hCG	LDH
Seminoma	0	+	++
Yolk sac tumor	+++	+	+
Choriocarcinoma	0	+++	+
Embryonal carcinoma	+	+	++
Teratoma	0	0	0

+++, Marker virtually always present in high amount and proportional to volume; ++, marker often seen in variable amount that is proportional to volume of disease; +, marker may be seen in variable amount, but not always; 0, never or seldom associated; AFP, alpha-fetoprotein; β-hCG, beta-human chorionic gonadotropin; LDH, lactate dehydrogenase.

Intro to BioMEMS

Purpose: Create a centrifugal lab-on-disk BioMEMS device that detects serum biomarkers for diagnosing germ cell tumors in testicular cancer

Why BioMEMS?

- Allows for quick, efficient, and less invasive method to detect blood serum biomarkers in the diagnosis and staging of germ cell tumors

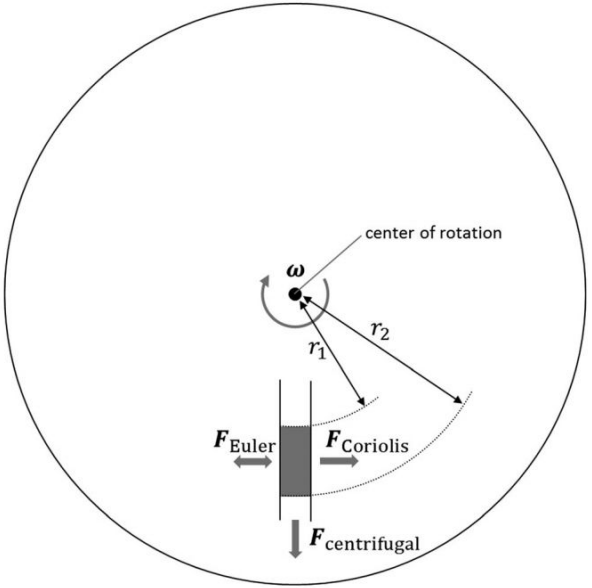
What theories & principles are at work?

- Centrifugal lab-on-disk, ELISA Assay, Fluorescent tagging and detection

Centrifugal Mechanism

- Takes advantage of different forces
- Intrinsic forces: due to presence or absence of centrifugation
  - Pseudo forces depend on disk radius and angular rotational frequency
    - centrifugal, Coriolis, Euler
  - Non-pseudo forces: viscous, pneumatic, capillary, fluidic inertia, drag
- Extrinsic forces: used when centrifugation alone is not enough
  - Paramagnetic beads for mixing
  - Pneumatic stirring
  - Electricity to aid separation

Pseudo forces involved in centrifugation



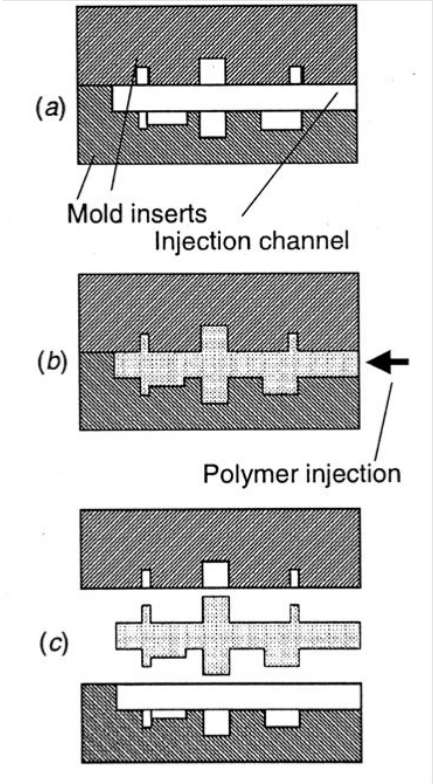
The Device

Fabrication Overview:

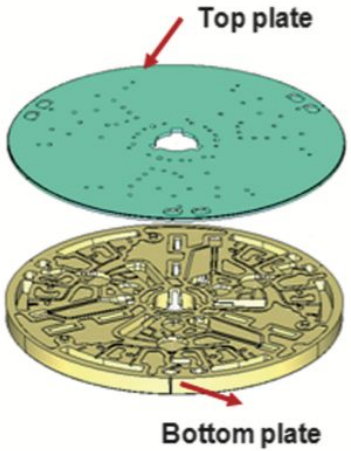
- Materials: PMMA film
  - Biocompatible material for microfluidics purposes
  - Similar biomechanical behavior as biological tissues
- PMMA is injection molded to form bottom and top plates
- UV adhesive applied and cured to seal plates together
- Appropriate use of microvalves to transfer sample amongst centrifugal device chambers

Fabrication of Centrifugal Device

- Device composed of top and bottom plate
- Injection molded plates of PMMA
- Top and bottom plates bonded using UV adhesive
- UV adhesive applied to bottom plate in pattern according to UV bonding image for device
- Top plate and bottom plate aligned, assembled and UV cured for 10 seconds
- Inlet holes present on top plate for sample



Injection Molding Process



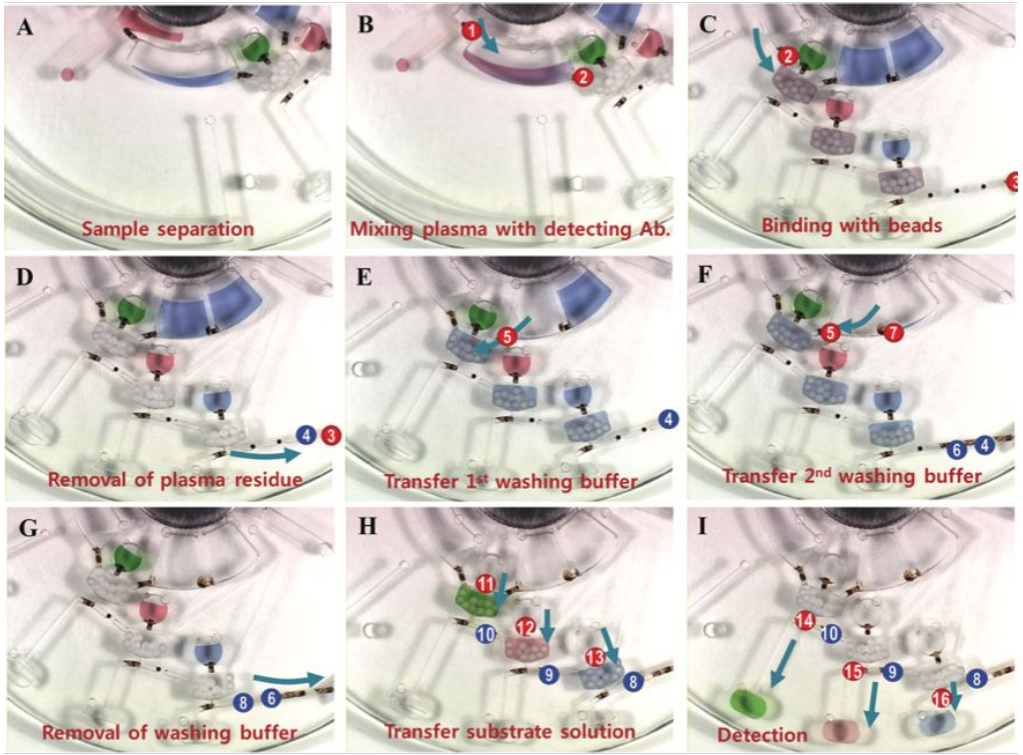
UV bonding image

Valve Actuation Strategy

- Valves control microfluidic transport through different chambers of device
- Valves include capillary and active valves
- Most common active valves in centrifugal systems involve paraffin wax, which can be melted easily
- Cho et. al report use of laser irradiated ferrowax microvalve (LIFM) in microfluidic platform for control of flow through the device

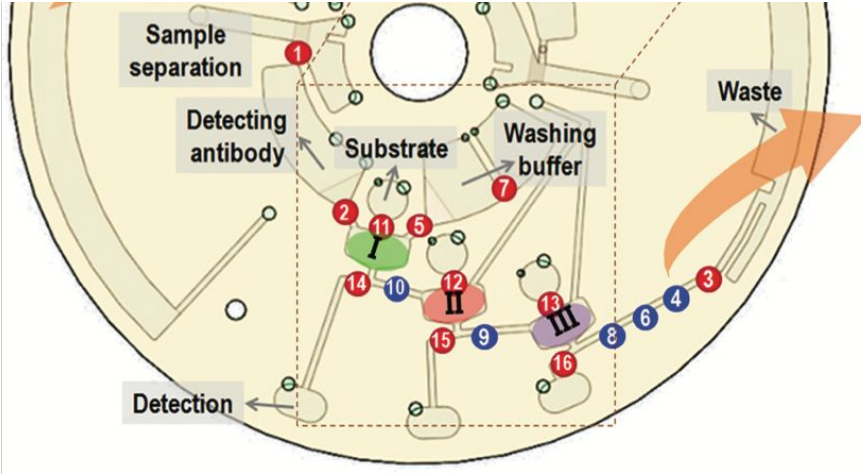
Flow Through centrifugal device

- Plasma is separated from blood elements through centrifugation
- Plasma transferred to reaction chamber where ELISA reaction occurs with mixing by centrifugation
- Sample transferred to detection chamber where fluorescence spectroscopy occurs

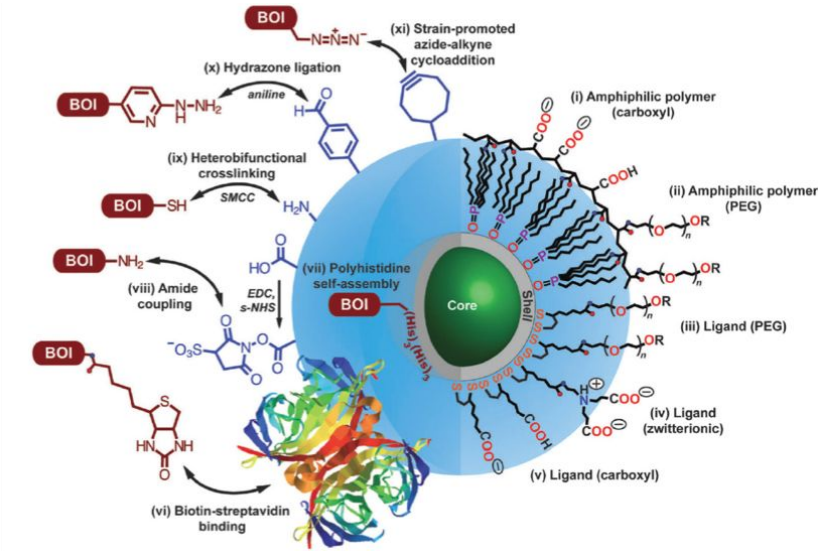


Reaction Chamber

- After blood separation, plasma is transferred into reaction chamber
- In reaction chamber, the analytes (LDH, hCG and AFP) bind to polystyrene labeled antibody with mixing occurring by clockwise and counterclockwise rotation
- QD labelled antibodies specific for target analyte-antibody complex bind to targets
- After ELISA reaction, sample is washed and transferred to the detection chamber



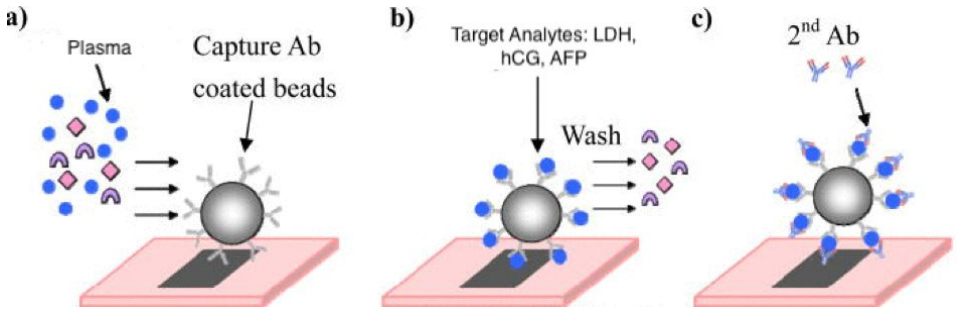
Capture protein and Detection protein



- Capture protein consists of covalent conjugation of anti-LDH, anti-hCG and anti-AFP to polystyrene beads (used for easy mixing and better separation with centrifugal forces)
- Detection protein consists of CdSe/ZnS core-shell quantum dots of varying crystal size fused to antibodies specific for the respective analyte antibody complex

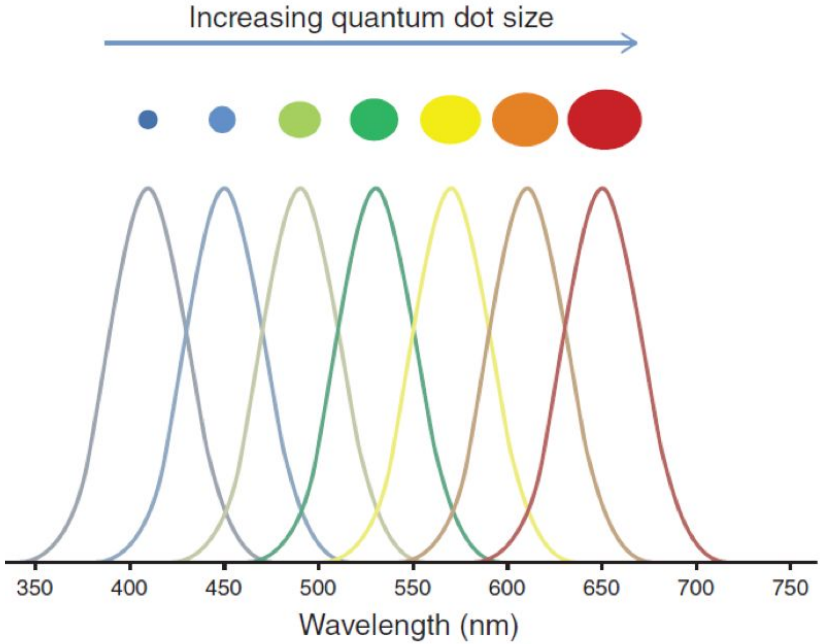
Sandwich ELISA

- PS beads conjugated to anti-LDH, anti-hcG and anti-AFP antibodies bind to target analyte within mixing chamber
- Secondary antibody fused to nanoparticle bind these conjugated protein complexes
- 3 different nanoparticles with different emission wavelengths (ie 480, 510, 540 nm) for simultaneous detection in detection chamber



Detection Chamber

- By varying QD crystal dimensions, emission spectrum is tunable
- Use of fluorescent spectrophotometry to detect LDH, hCG and AFP labelled with QDs
- Single excitation wavelength (330 nm) required for CdSe/ZnS core-shell QDs
- Emission spectrum at 480, 510, 540 nm detected



Testing

- Compare microfluidic device measured concentrations for LDH, hCGP and AFP with standard lab assessment used currently
- Compare with control (cancer free patients)
- Obtain lowest serum concentration detectable to assess ability to detect cancer recurrence and/or track therapy during cancer

Limitations

- Depends on appropriate separation of blood components, specific binding of primary and secondary antibody, washing and appropriate tracking of sample though microfluidic device
- Patient intervariability in serum concentration of serum biomarkers and their correlation with cancer presence (though limitation with currently used methods as well)

Conclusion

Using the techniques of centrifugal lab-on-disk, Sandwich ELISA, and fluorescent tagging and detection, we were able to design a microfluidics device for the staging and prognosis of Testicular germ cell tumors. This allows for a faster, more portable, and less invasive method to detect biomarkers important in the diagnosis of testicular cancer than methods currently used in clinic.

Future uses:

- Apply device to other Cancer detection and diagnosis
- Used to detect any serum proteins

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Questions?