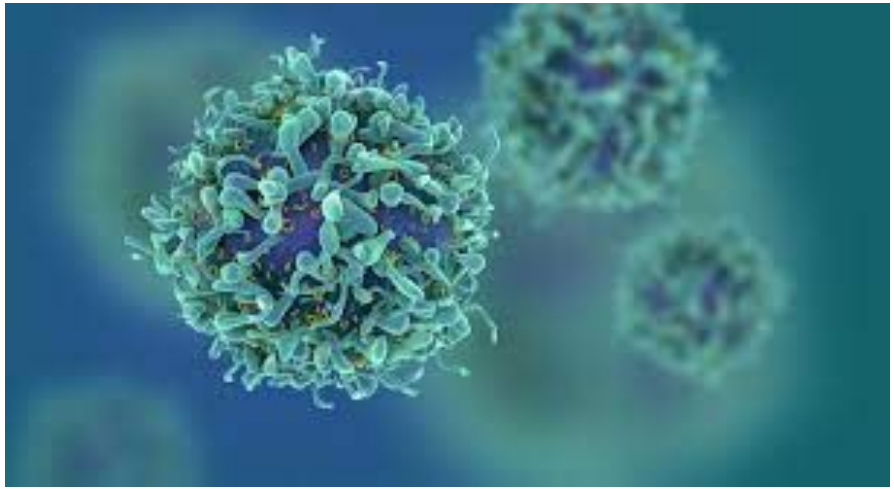
Small but mighty: Creating an organ-on-a-chip model to study nanoparticle extravasation and uptake at tumor sites

Katelyn, Teresa, Theodore, and Natalie



How can nanoparticles be used to treat cancer?

- Nanoparticles can deliver cancer therapeutics in a targeted manner with controlled release.[1]
- Often coated with targeting moieties which serve as ligands for receptors overexpressed on cancer cells.[1]
    - Folate, aptamers, peptides, antibodies
- Some nanoparticles rely on “passive targeting” to reach cancer cells by relying on their size/shape.[1]

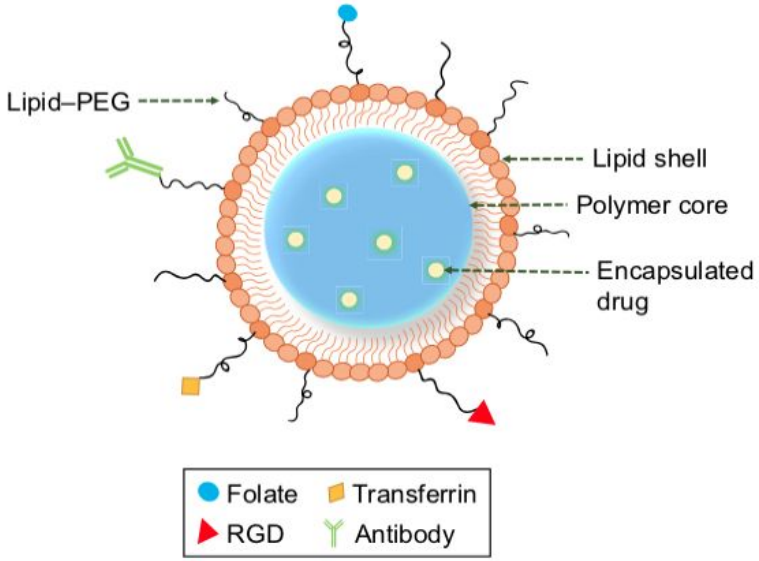


Fig. 1: Diagram of general therapeutic nanoparticle architecture.[1]

Nanoparticles and Tumor Vasculature

- Tumors tend to rapidly initiate angiogenesis for oxygen/nutrients supply, but these blood vessels are often leaky.[2]
- Tumors tend to have poorly developed lymphatic systems.
- These features together create the "Enhanced Permeability and Retention (EPR) Effect."[2]
- Nanoparticles tend to extravasate from circulation more easily at tumor sites due to leaky vessels and can accumulate more rapidly due to limited lymphatic drainage within the stroma.[2]

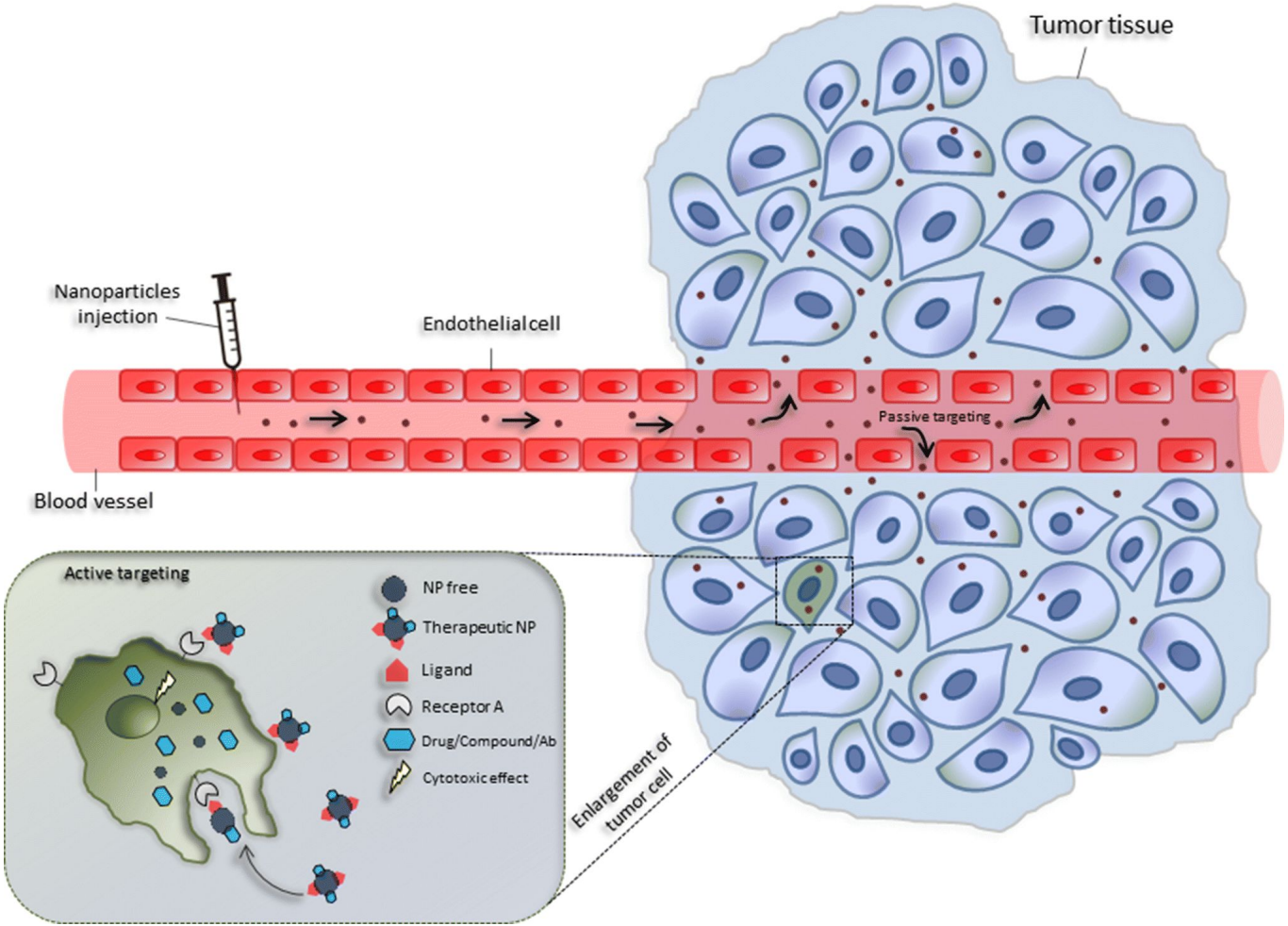
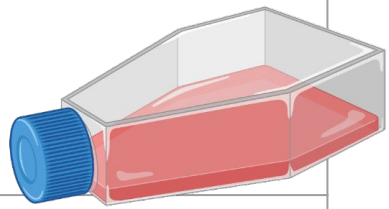
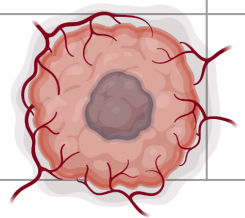


Fig. 2: Schematic of nanoparticle extravasation and active targeting of cancer cells.[2]

Challenges with Nanoparticle Studies and Current Need

Current Approaches	Challenges
Animal models [5] 	• Differences in the tumor microenvironment of animal models compared to humans. ○ Mouse vessels are more permeable compared to human vessels • High cost and long testing periods.
2D/3D static cell culture [6]	• Limited capability in recapitulating in vivo tumor microenvironment • Lack of chemical gradients and flow conditions • Does not represent heterogeneity across patients 
Leveraging EPR [5] 	• Variability between different tumors and even within the same tumor ○ Not all cancers present permeable vessels

Previous Research on Microfluidics and OOC in Cancer [6]

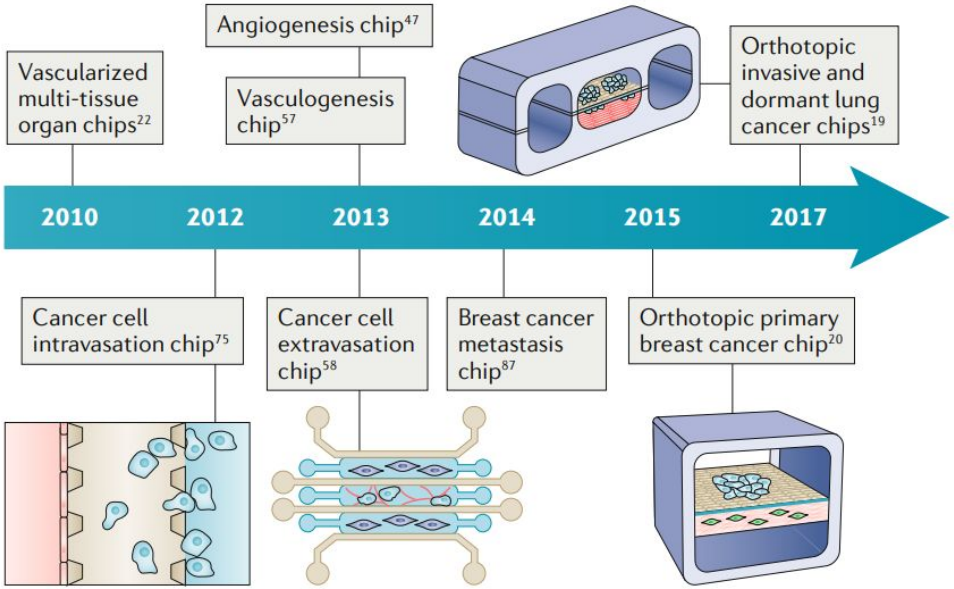
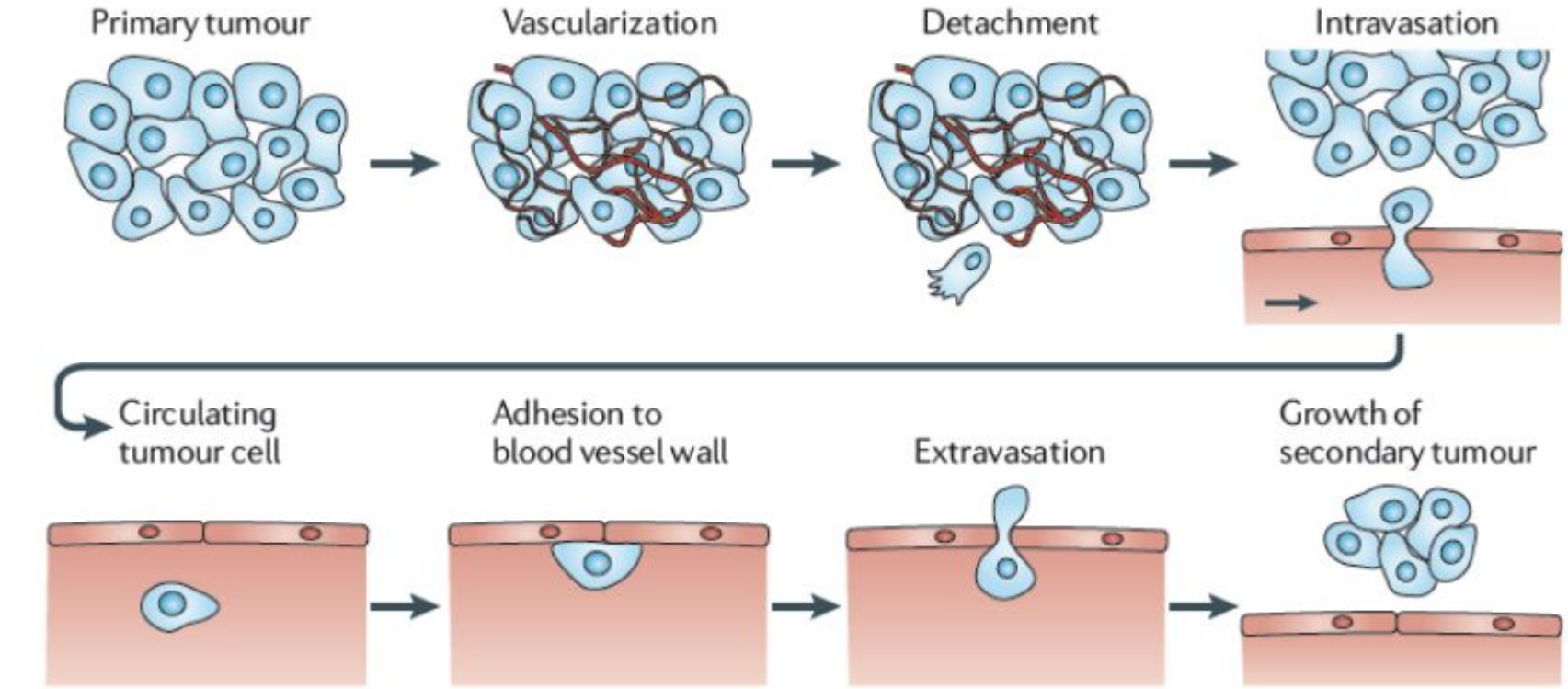


Fig. 1 | **A timeline showing the development of different cancer organs-on-chips.**

- OOC can mimic disease states
- Areas used in cancer research
  - Steps of cancer cascade
  - Tumor growth
  - Angiogenesis
  - EMT
  - Role of surrounding cells/env.
- Organ responses to
  - Drugs
  - Toxins
  - Radiation
  - Pathogens
  - Immune system

Previous Research on Microfluidics and OOC in Cancer

Chen et al. studied extravasation of cancer cells using a



- TNF-α increased permeability by ~5x

Proposed BioMEMs Solution

- We propose an OOC with 2 primary chambers which models a capillary and its surrounding tumor microenvironment.

Upper Chamber:

- Inlet/outlet flows and pressure mimic a capillary, suspended nanoparticles flow through
- Lined with endothelial cells

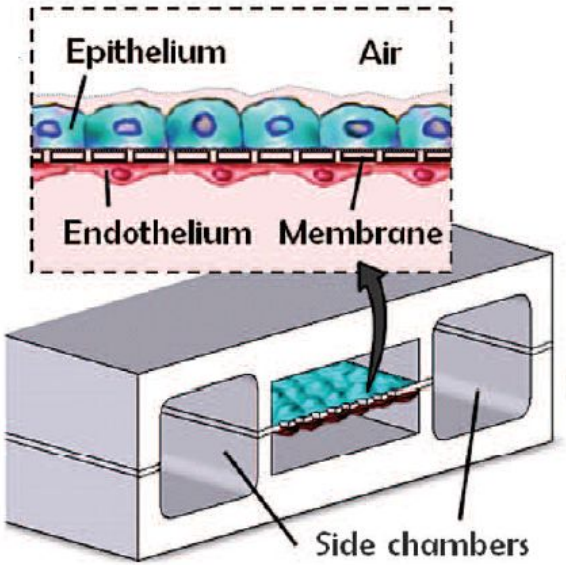
Chamber Barrier:

- PDMS membrane with variable porosity coated with ECM proteins for cell adhesion.

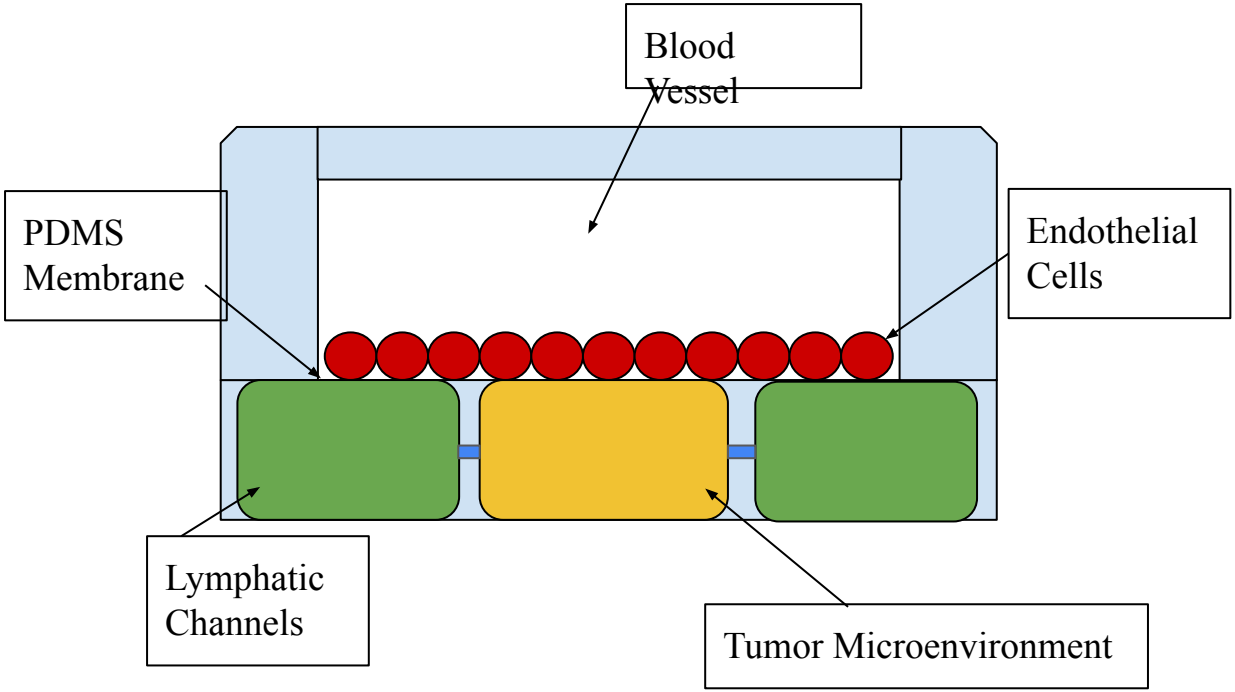
Lower Chamber:

- Inlet/outlet flows and pressures are variable for a tunable tumor environment
- Collagen matrix seeded with cancer and other relevant cells depending on modeling desires.
- Two side channels represent lymphatic drainage (can be manipulated for high/low levels of drainage)

Schematic of Existing "Lung-on-a-chip" Model [3]



Proposed Tumor Microenvironment OOC



Fabrication [6]

1) Silicon wafer with SU-8

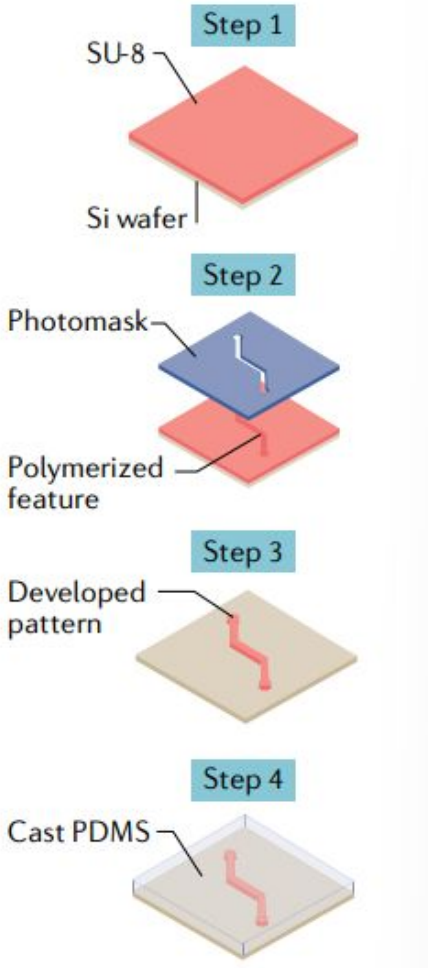
2) Position photomask with channel pattern and expose mask/resist to UV light

3) “Develop” to dissolve un-polymerized SU-8

4) Cast PDMS

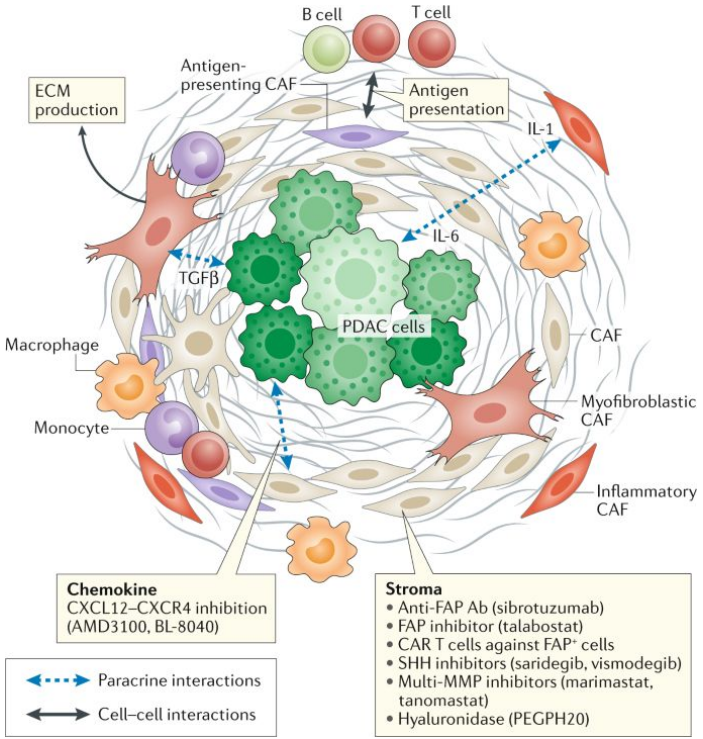
5) Repeat Steps 1-4 to create both top and bottom chambers

6) Insert porous PDMS membrane between the PDMS channel chambers and bond after plasma oxidation



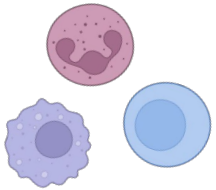
Seeding Cells and Modulating Permeability

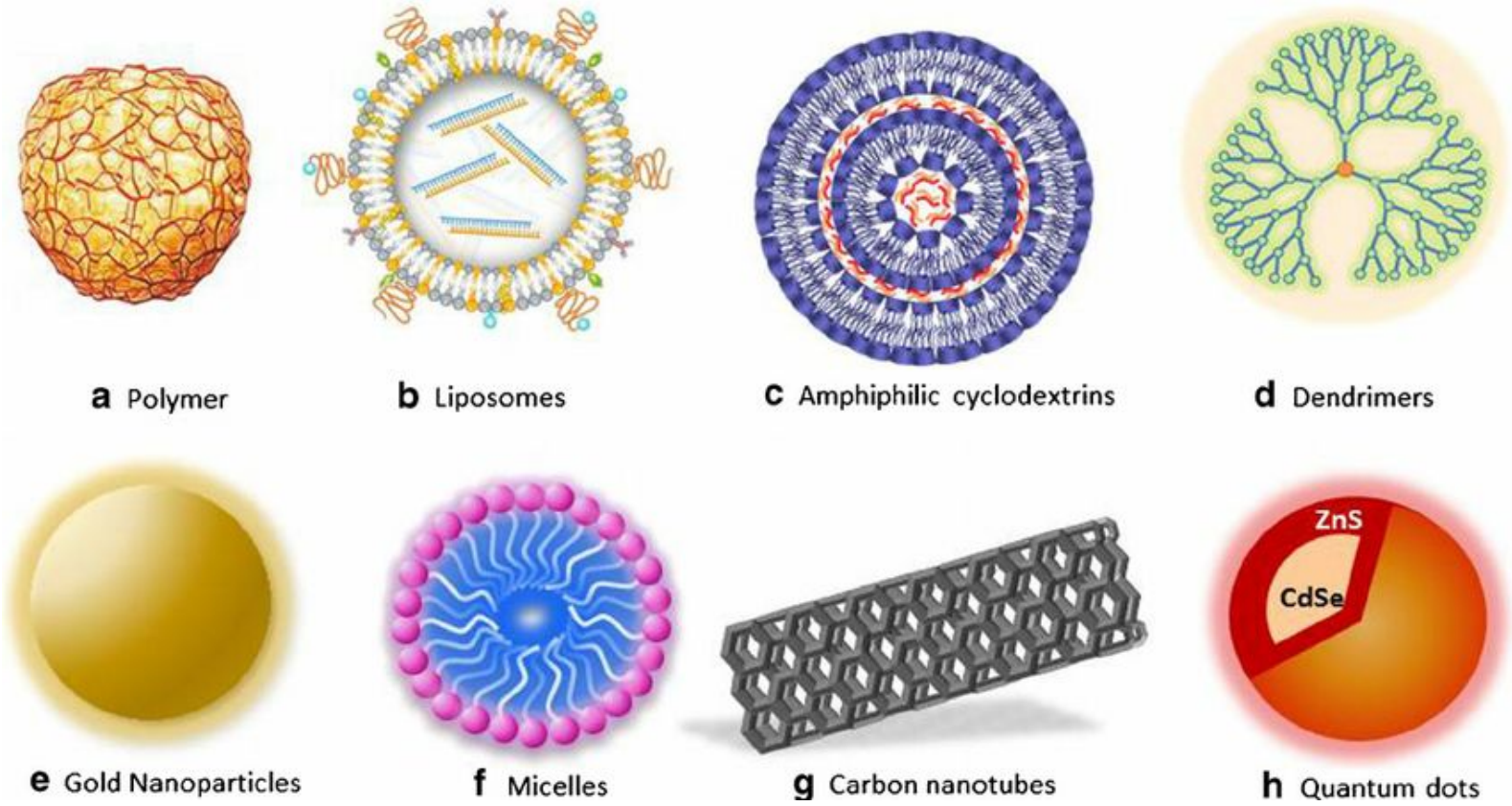
- Before cell seeding, the porous PDMS membrane is coated with ECM
  - Could be composed of collagen, laminin, fibronectin, etc
- Endothelial Cells (HUVECs) seeded in the upper chamber via perfusion with shear stress
  - Permeability can be modulated by delivering signals like VEGF, TNF-alpha, and fibrinogen. [4]
- The lower chamber's cancer microenvironment consists of cancer cells, fibroblasts, and other relevant cells cultured in a collagen hydrogel



Biocompatibility

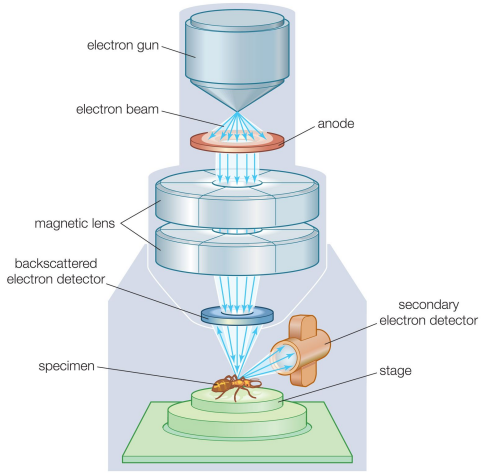
- This device will not be placed in the body, it merely serves as a (hopefully improved) *in vitro* model, so its biocompatibility with the body is not a concern
- PDMS is known to be highly compatible for mammalian cell culture [7]
- Media will be continually perfused to maintain cell viability

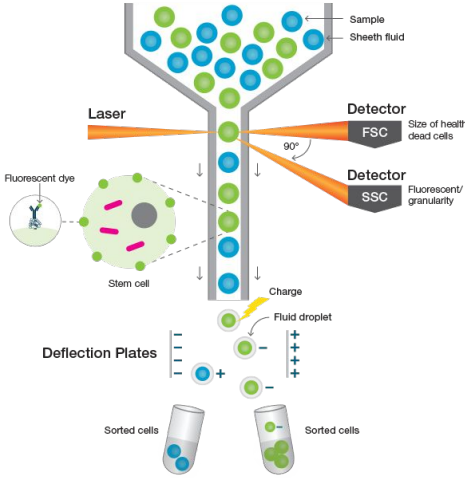
Tuning Factor	Variables	Impact
PDMS pores 	• Quantity • Density • Size	Modulates permeability of modeled capillary
Endothelial layer 	• Cell type • Growth factor	Extravasation/transcellular uptake of nanoparticles
Extracellular matrix 	• Protein composition	Stiffness/density of tumor microenvironment; nanoparticle distribution
Immune system 	• Surveying immune cell types in lower chamber	Observing immune response to nanoparticles
Interstitial pressure 	• Flow pressure through chamber • Lymphatic channels	Diffusion of nanoparticles in various cancer microenvironments can be observed

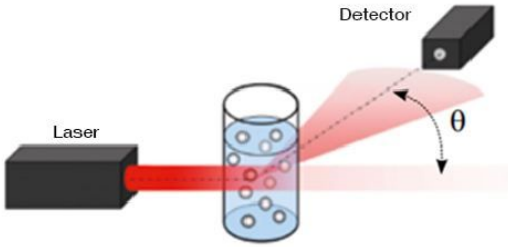


Ideally we will use this device to test a variety of nanoparticles with different features (e.g. materials, coatings, targeting moieties, size, shape)

Testing







Test Method	Transmission electron microscopy (**TEM**) and scanning electron microscopy (**SEM**)	Fluorescence activated cell sorting (**FACS**)	Dynamic light scattering (**DLS**)
Application	Visualization of nanoparticle structure, morphology and dispersion	Quantitative identification of nanoparticle internalization	Determination of nanoparticle aggregation
Advantages	High resolution	Multiparameter separation	Real time measurement
Disadvantages	Sample preparation	Needs cell suspensions	Sensitivity to solvent viscosity

Limitations

Other effects on nanoparticles

- Renal system filtering
- Immune system (macrophages)

Difficulty representing certain cancer types

- Hypovascularization of pancreatic cancer

Cost and resources for device fabrication and maintenance

Benefits and Future Directions

- Provides a cheaper, more tunable, and less variable solution than animal models
- Representativeness
  - Tune ECM and seeded cancer cells to recapitulate tumor microenvironment specific to different cancers
  - Tune to fit different patients
- Could also be used for cancer cell extravasation models
- Incorporate more immune response elements
  - Different macrophages
- Improve microfabrication techniques to allow for more universal use

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