



Technical Support: 3D Perfusion Bioreactor Assembly

*Add Extra **D**imension to Your **I**nnovation™*



General Overview: *Bioreactors*

Bioreactor Classification

Transport Dynamics

- Stir Bioreactors
- Rotating Flask Bioreactors
- Shaker Bioreactor
- **Perfusion Bioreactors**
- Magnetic Bioreactors

Cell Growth Carrier

- Liquid Phase
- Porous Beads
- Solid Beads
- **Polymer Scaffolds**
- Hollow Fibers



Perfusion Bioreactor: *Applications*

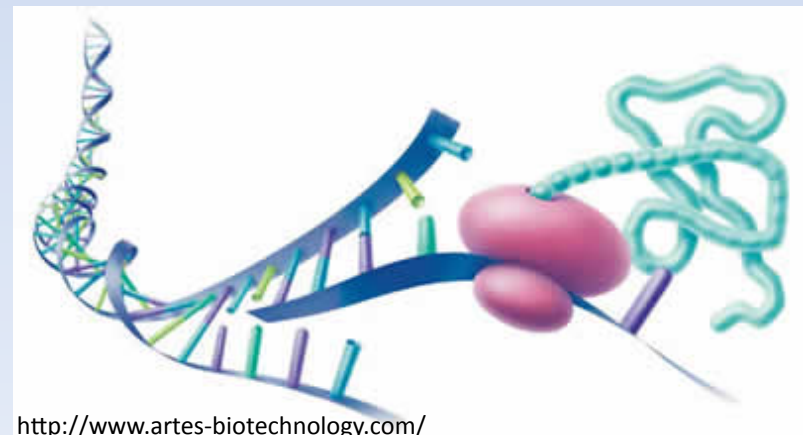
□ Tissue Engineering

- Bone
- Cartilage



□ Scaling-up Processes

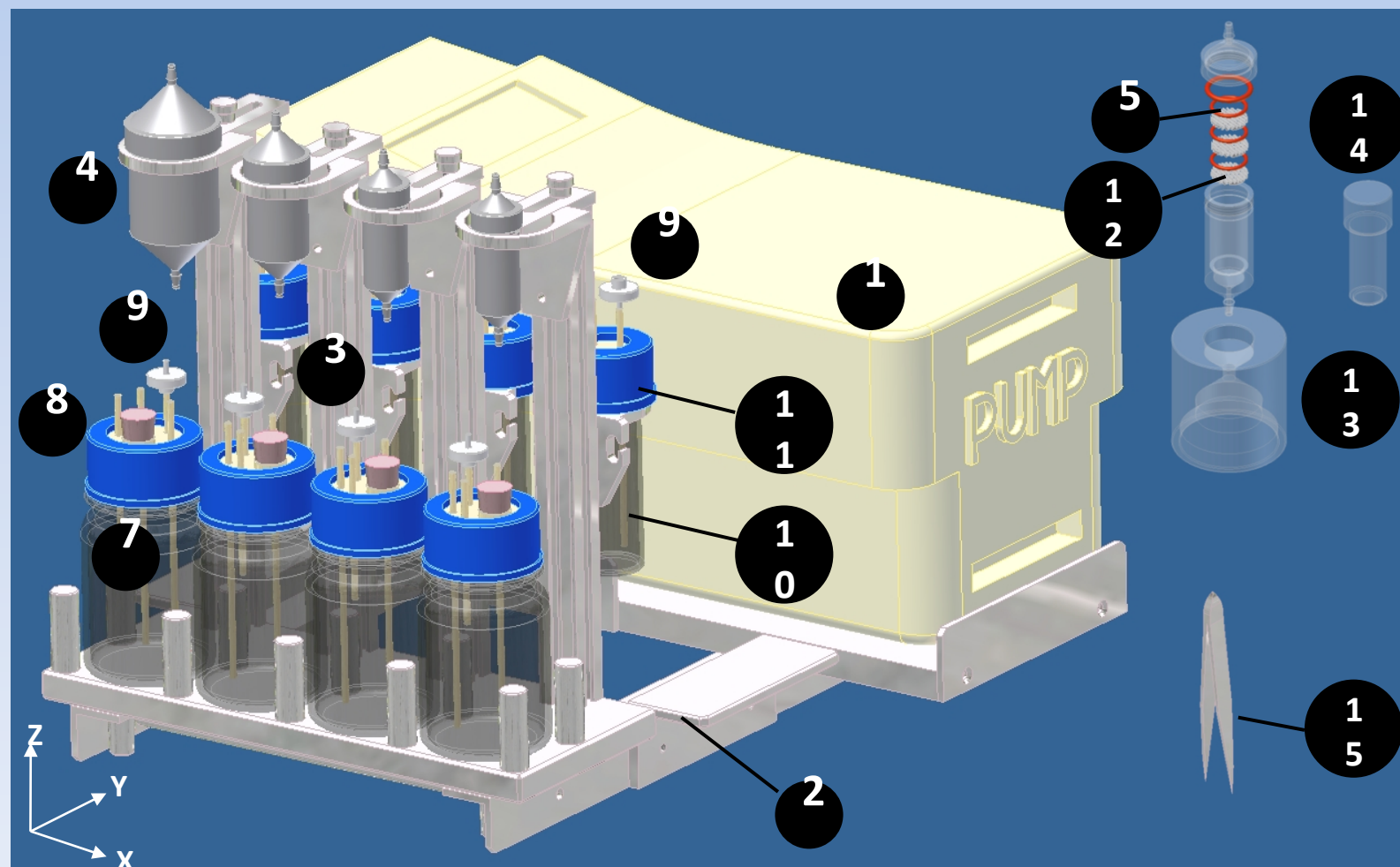
- Recombinant Protein Production
- ECM-Coated Scaffolds
- General Cell Culture



<http://www.artes-biotechnology.com/>

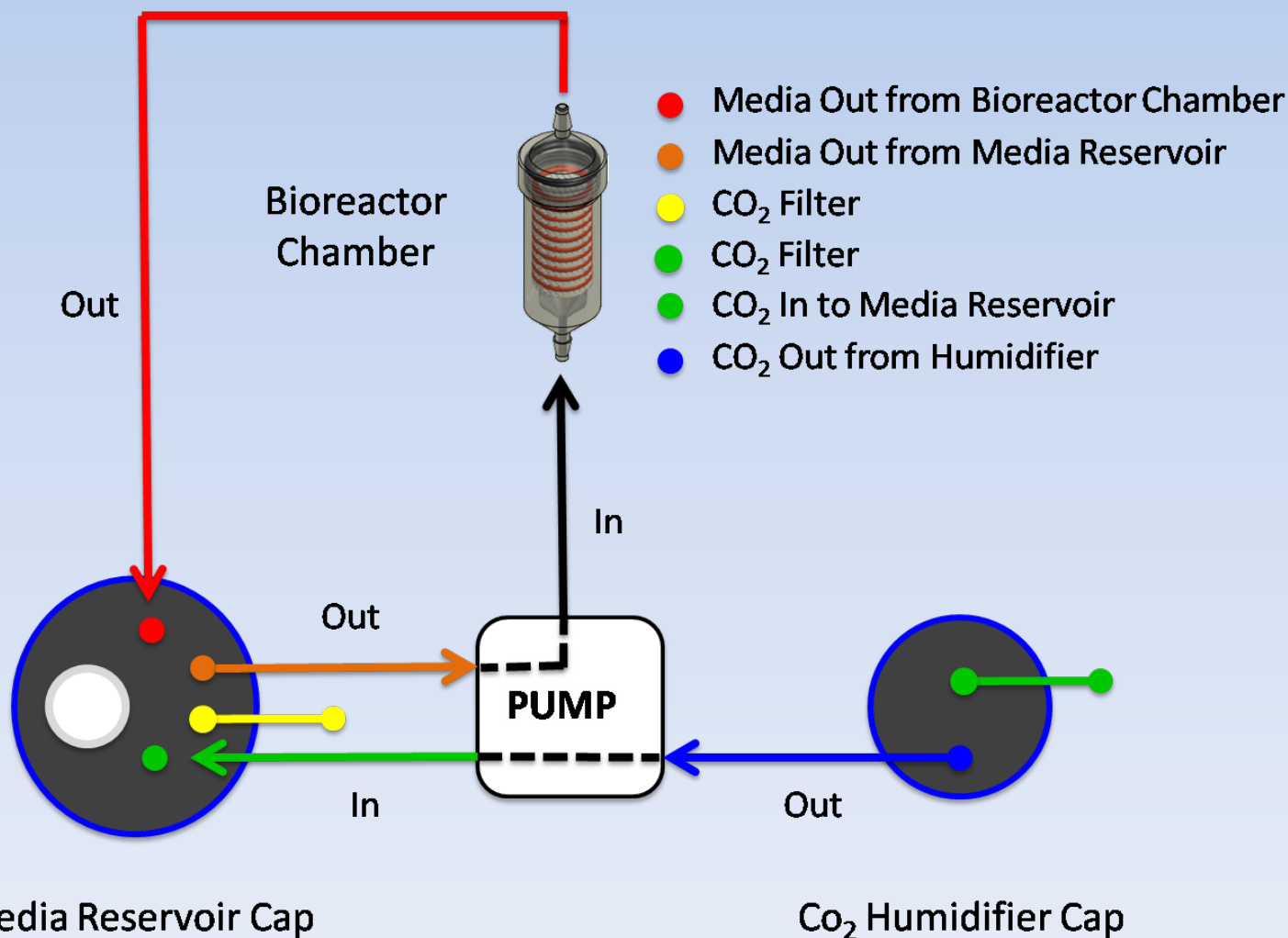


3D Perfusion Bioreactor: *General*





3D Perfusion Bioreactor: *Specific*



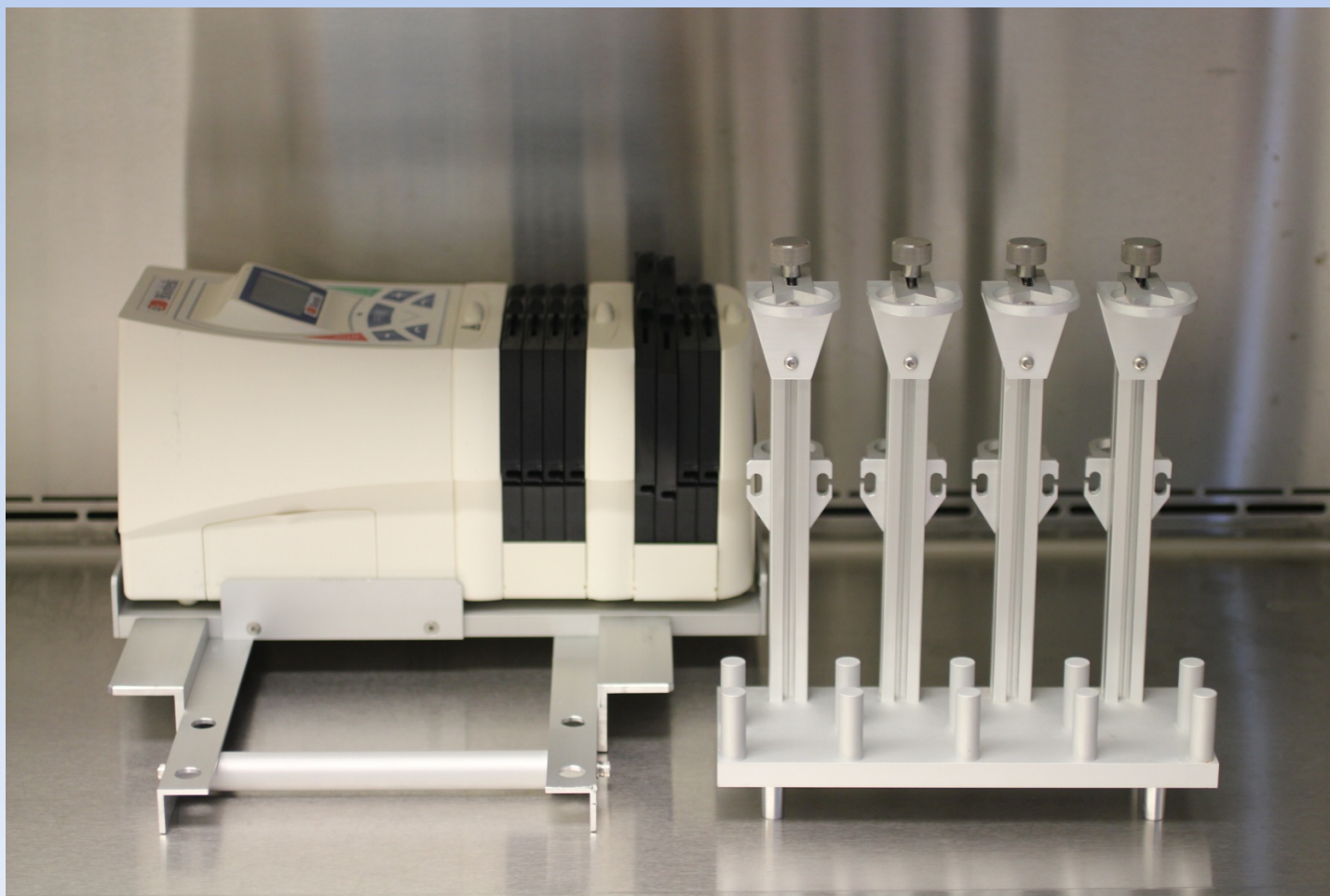


Bioreactor Functional Parts





I: Metal Frame and Pump

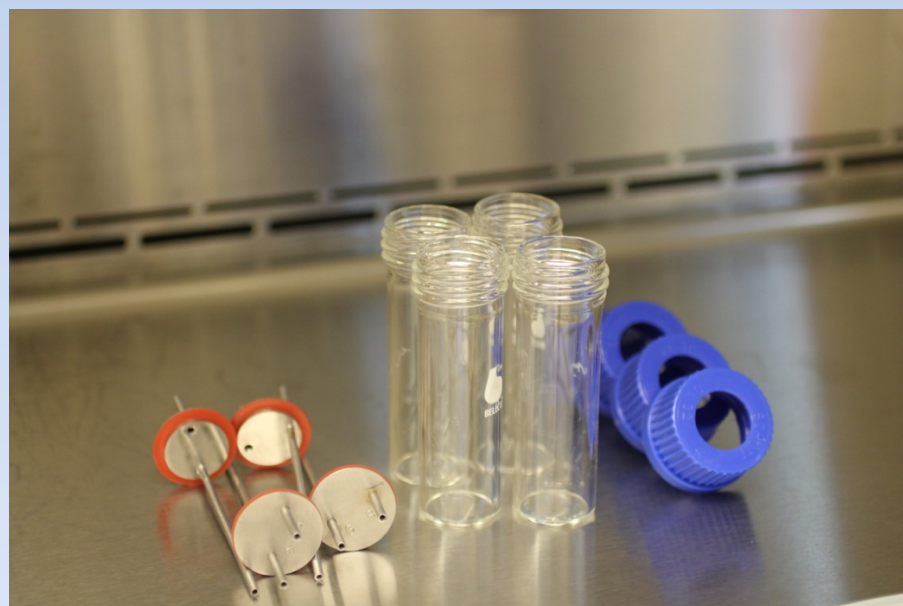




II: Media and Humidifier Bottles



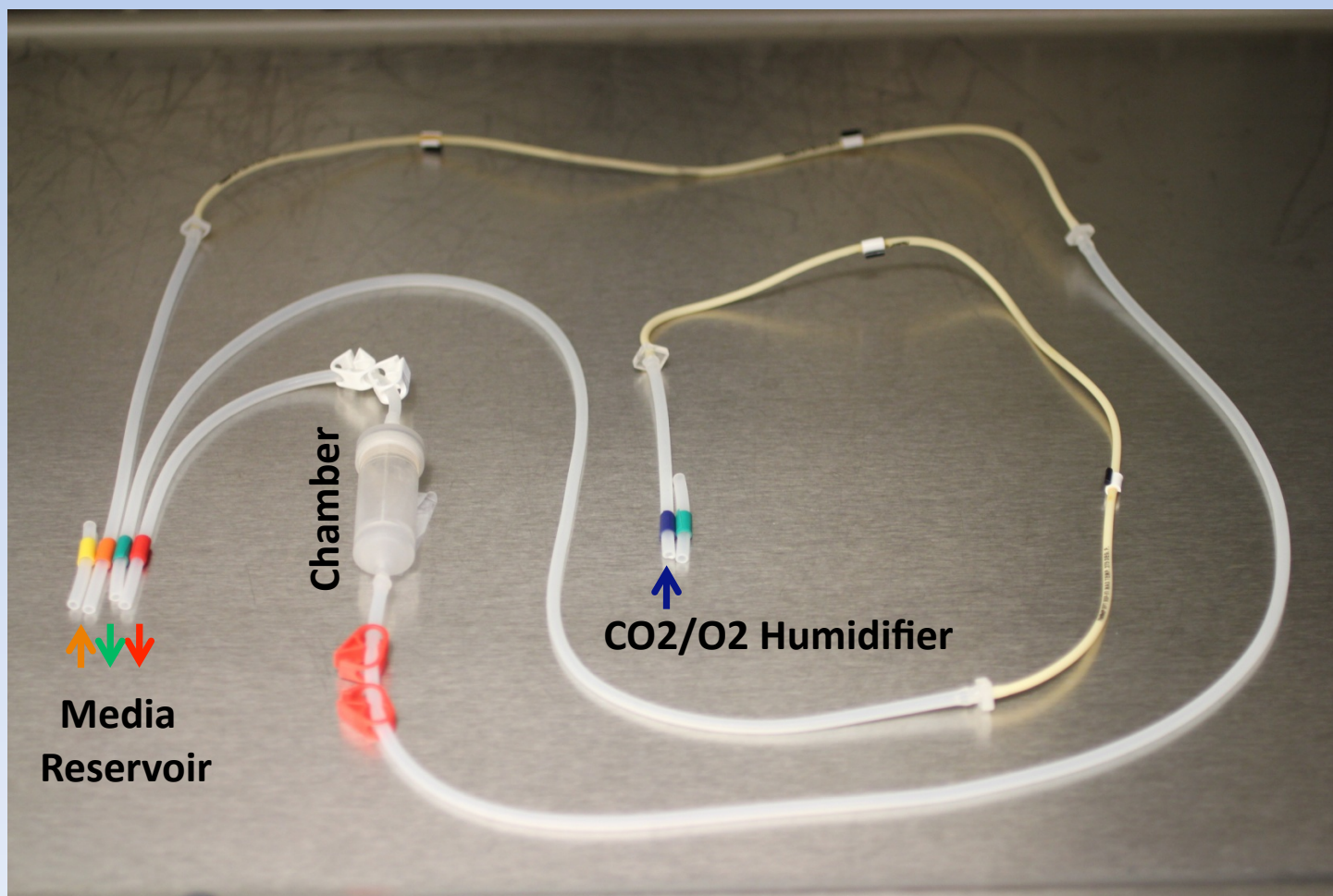
Media Bottles



Humidifier Bottles



III: Media and Gas Perfusion Tubing



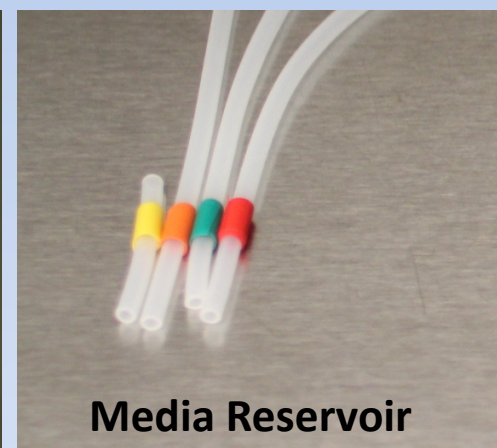
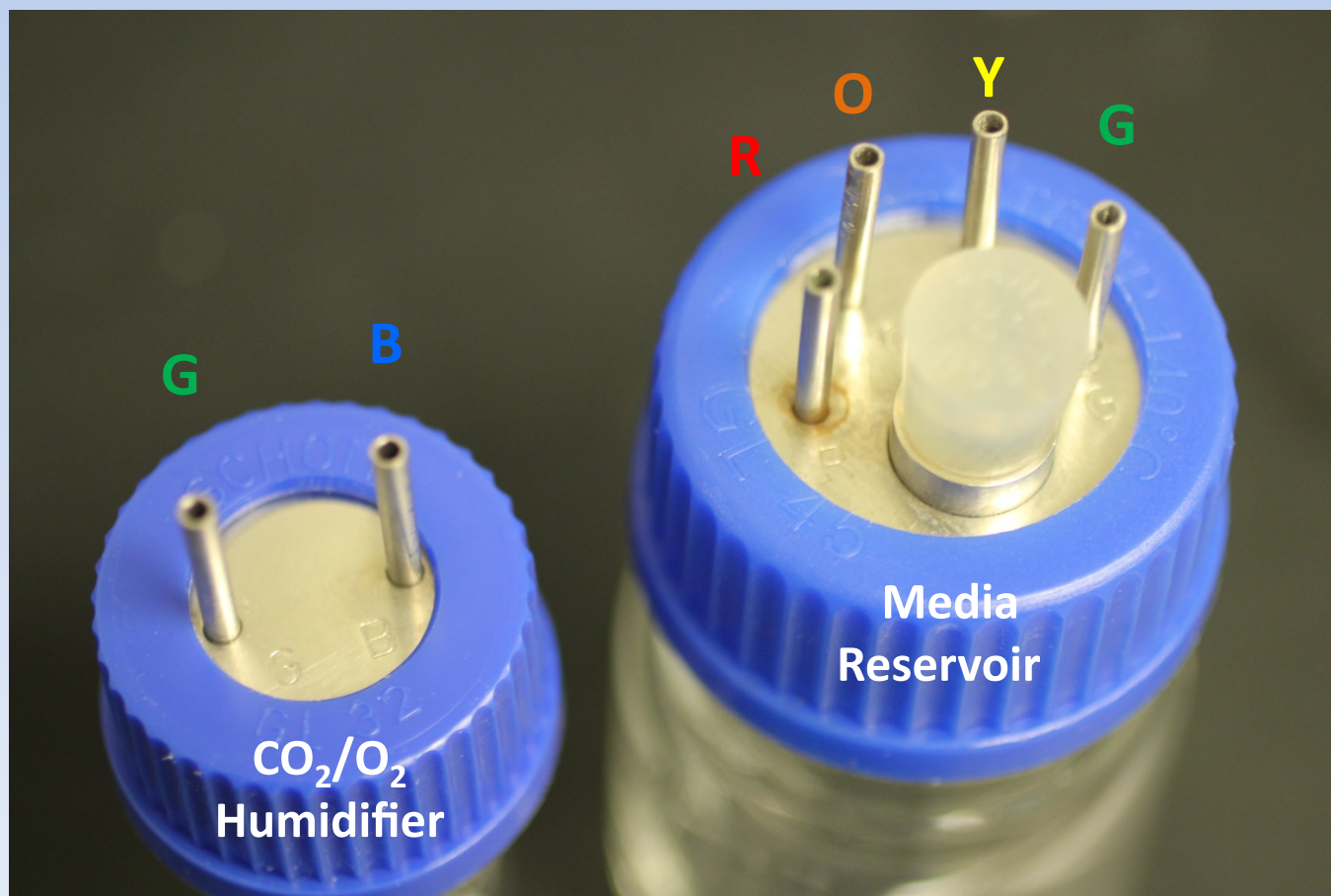


Tubing Assembly and Media Equilibration



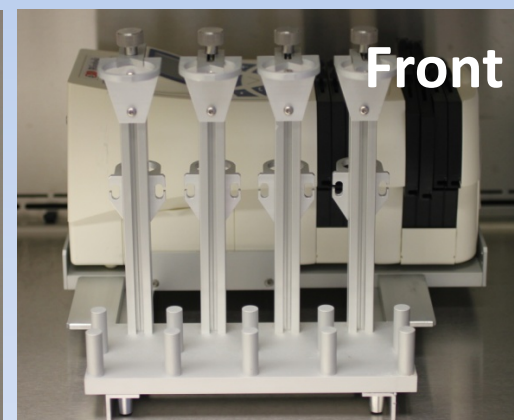
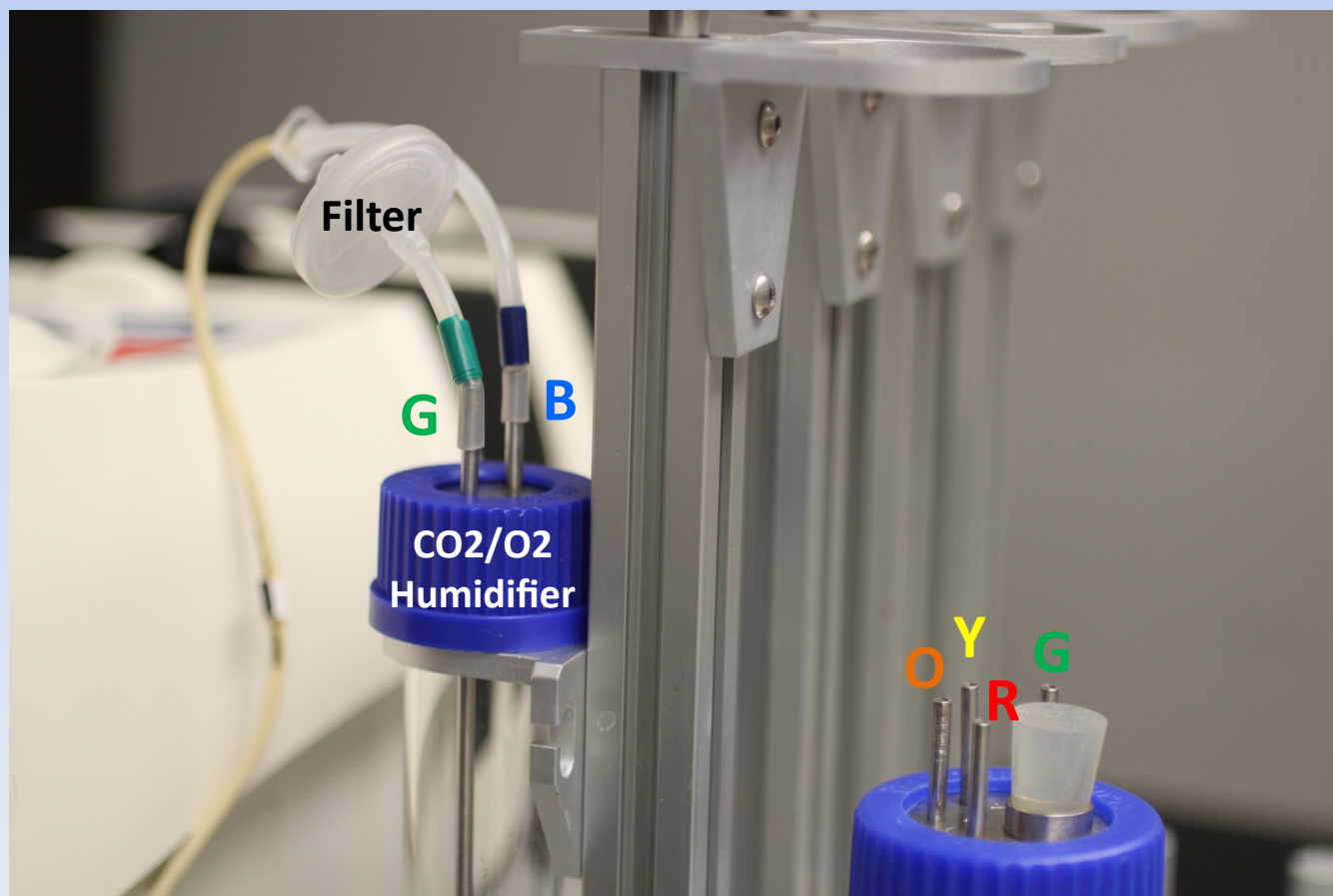


Color Coordinated Tubing Assembly





Step 1: CO_2/O_2 Perfusion



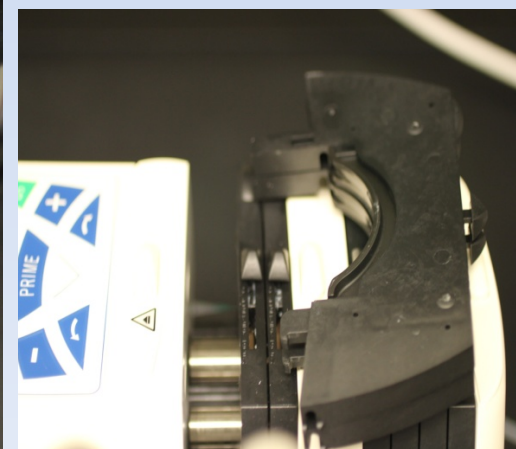
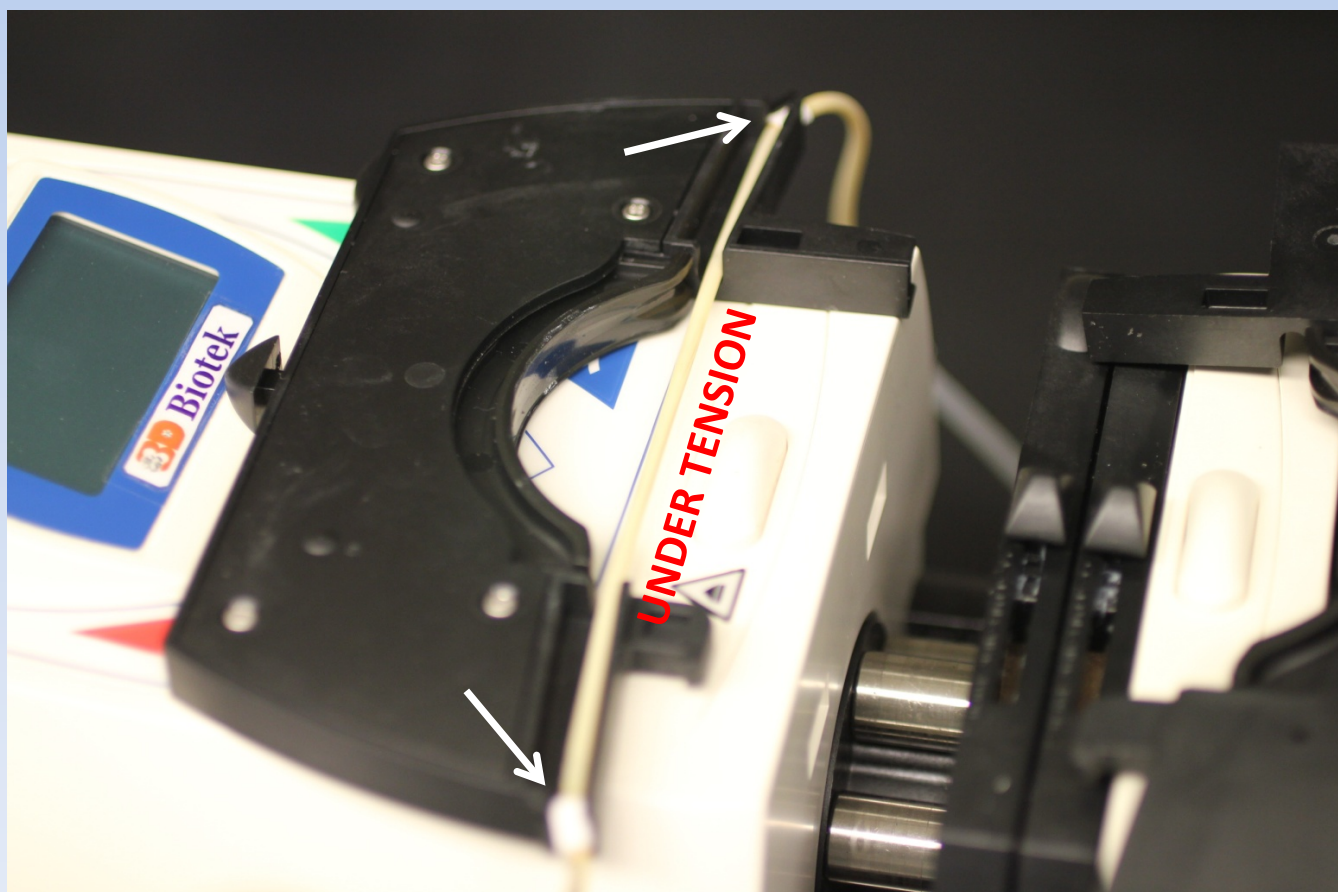
Front



Back

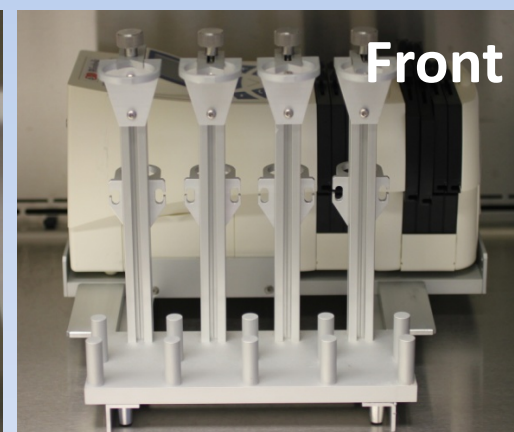
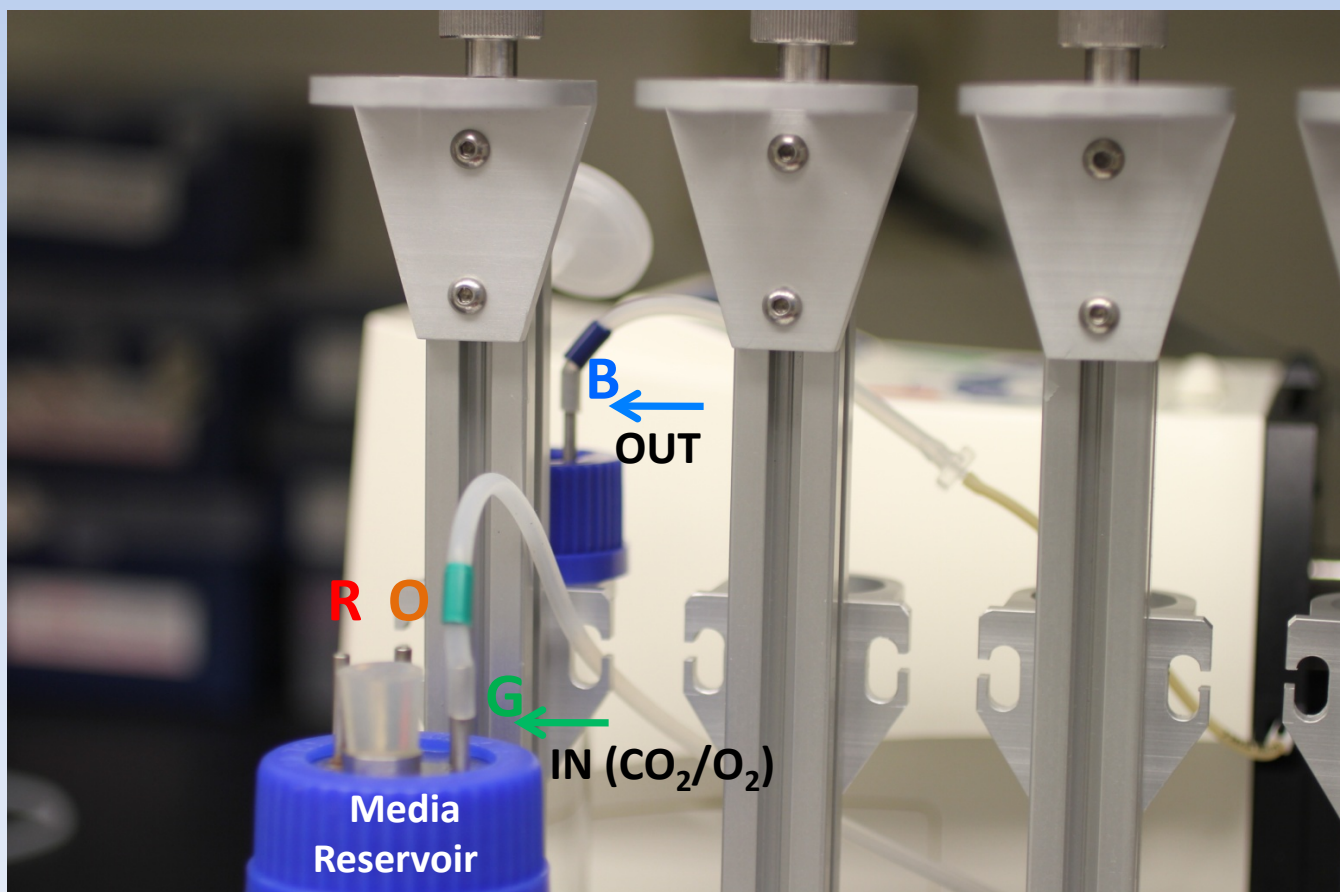


Step 2: CO_2/O_2 Perfusion



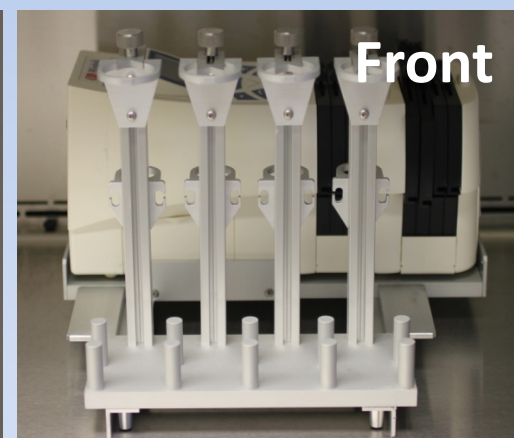
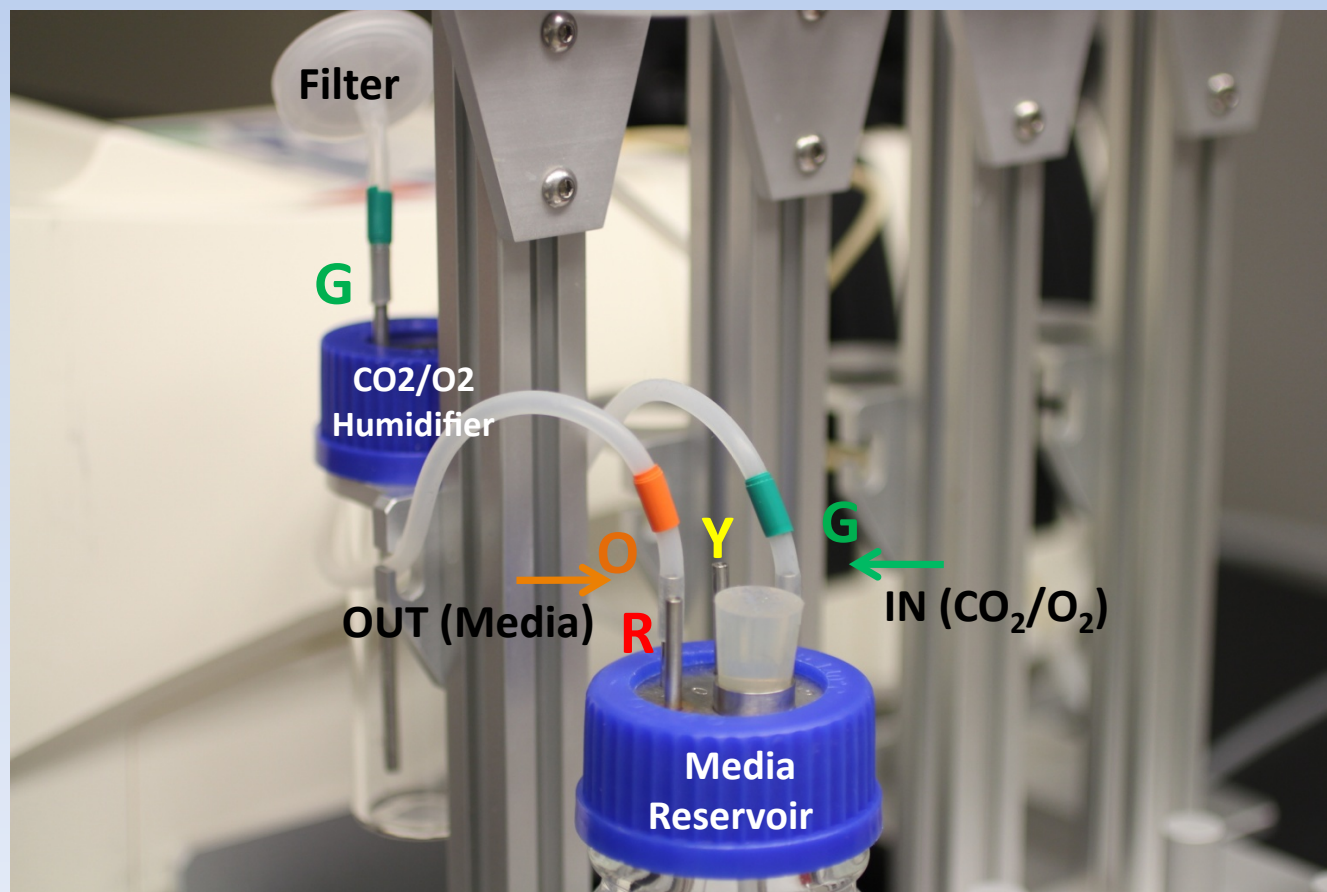


Step 3: CO_2/O_2 Perfusion



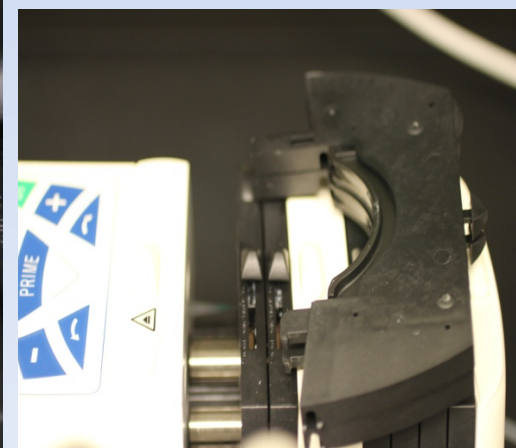
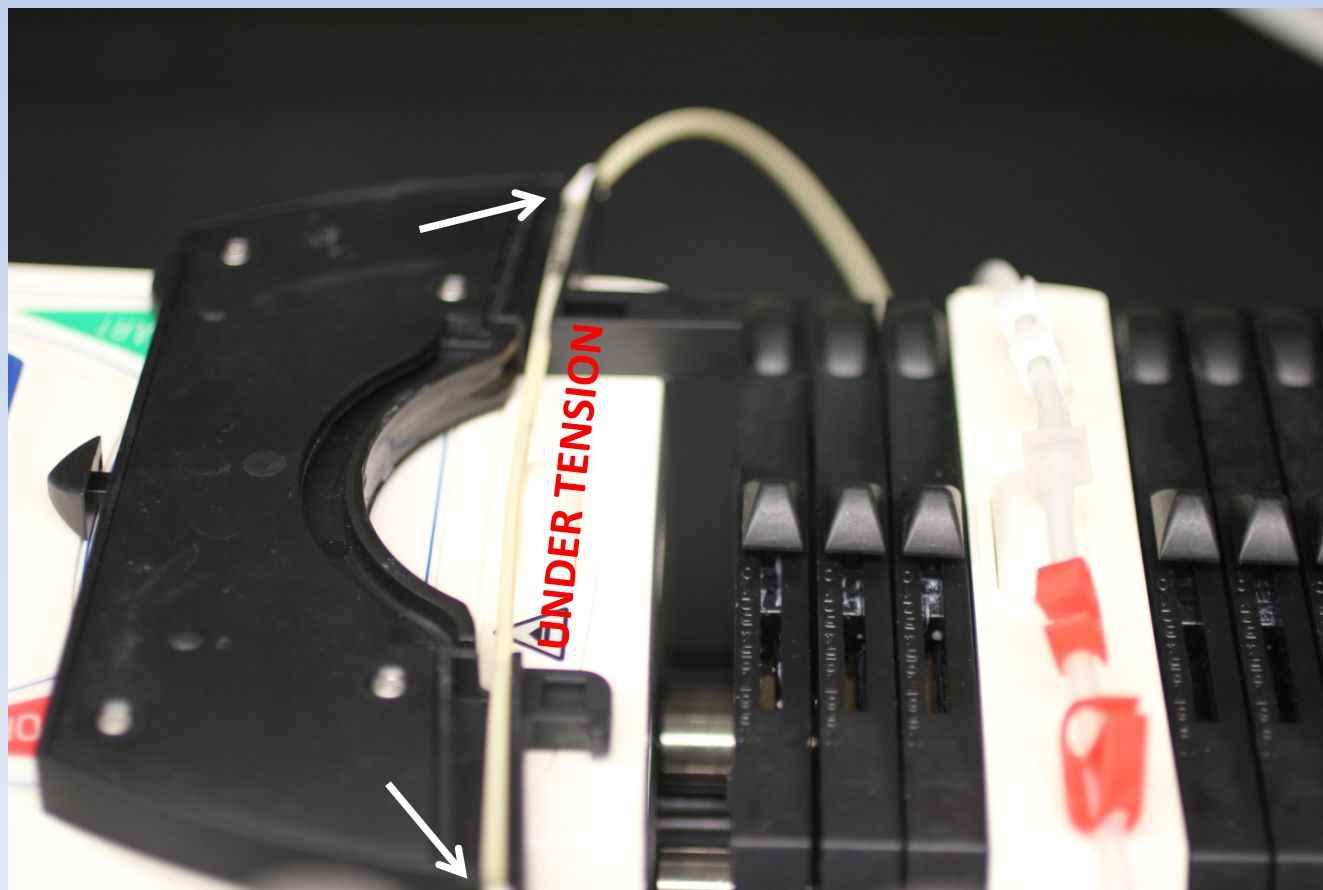


Step 1: Media Perfusion



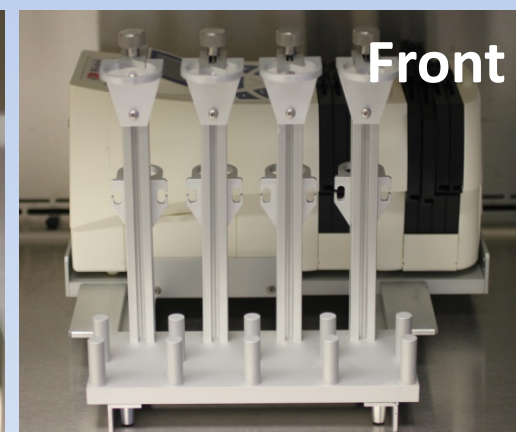
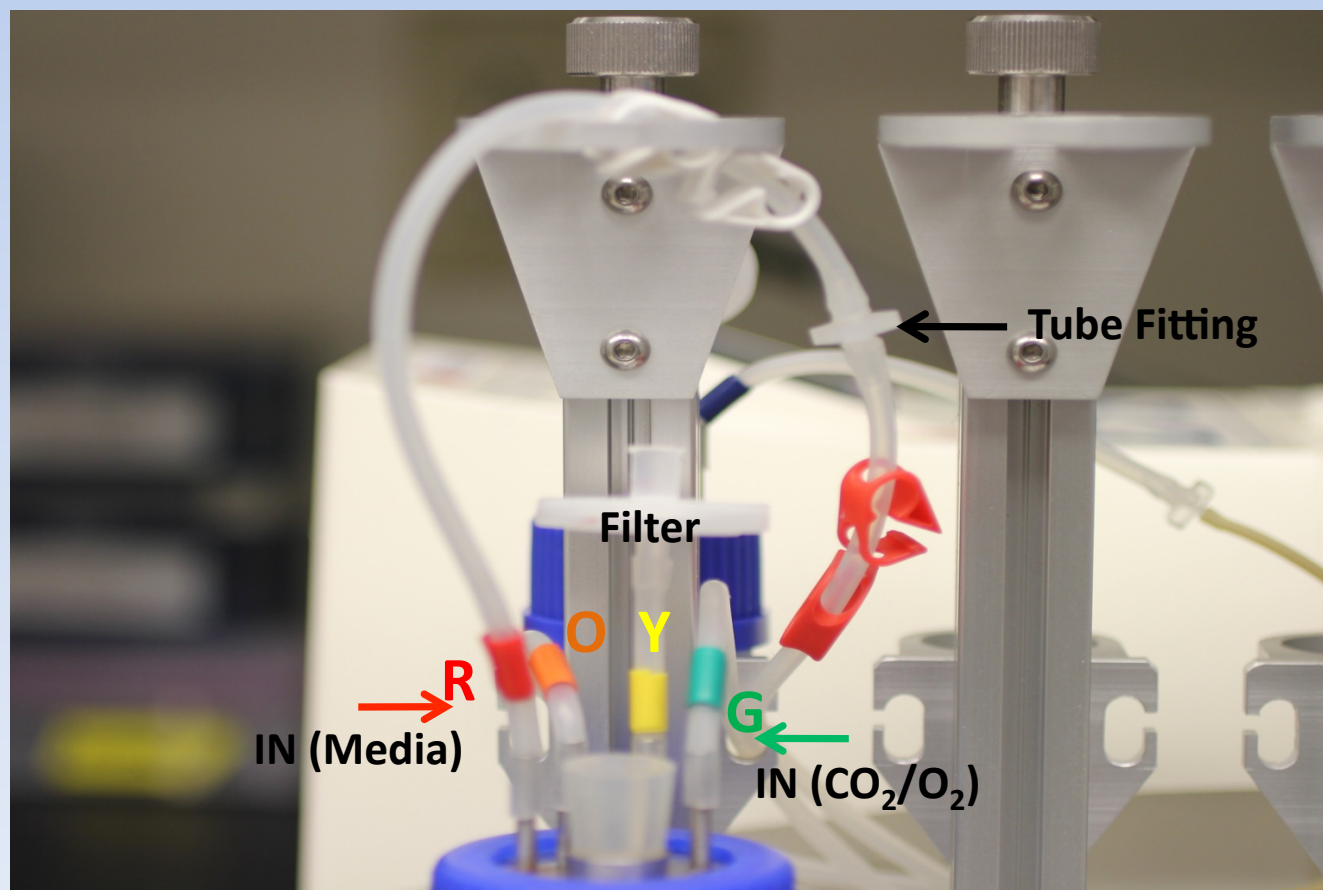


Step 2: *Media Perfusion*





Step 3: Media Perfusion





Bioreactor Chamber and Tubing Volumes

Bioreactor Format and Tubing	Volume to Fill (ml)
6 –Well Chamber	45-ml
12-Well Chamber	15-ml
24-Well Chamber	7.0-ml
Media Tubing	5.0-ml

Reservoir Bottle Max. volume 90-ml

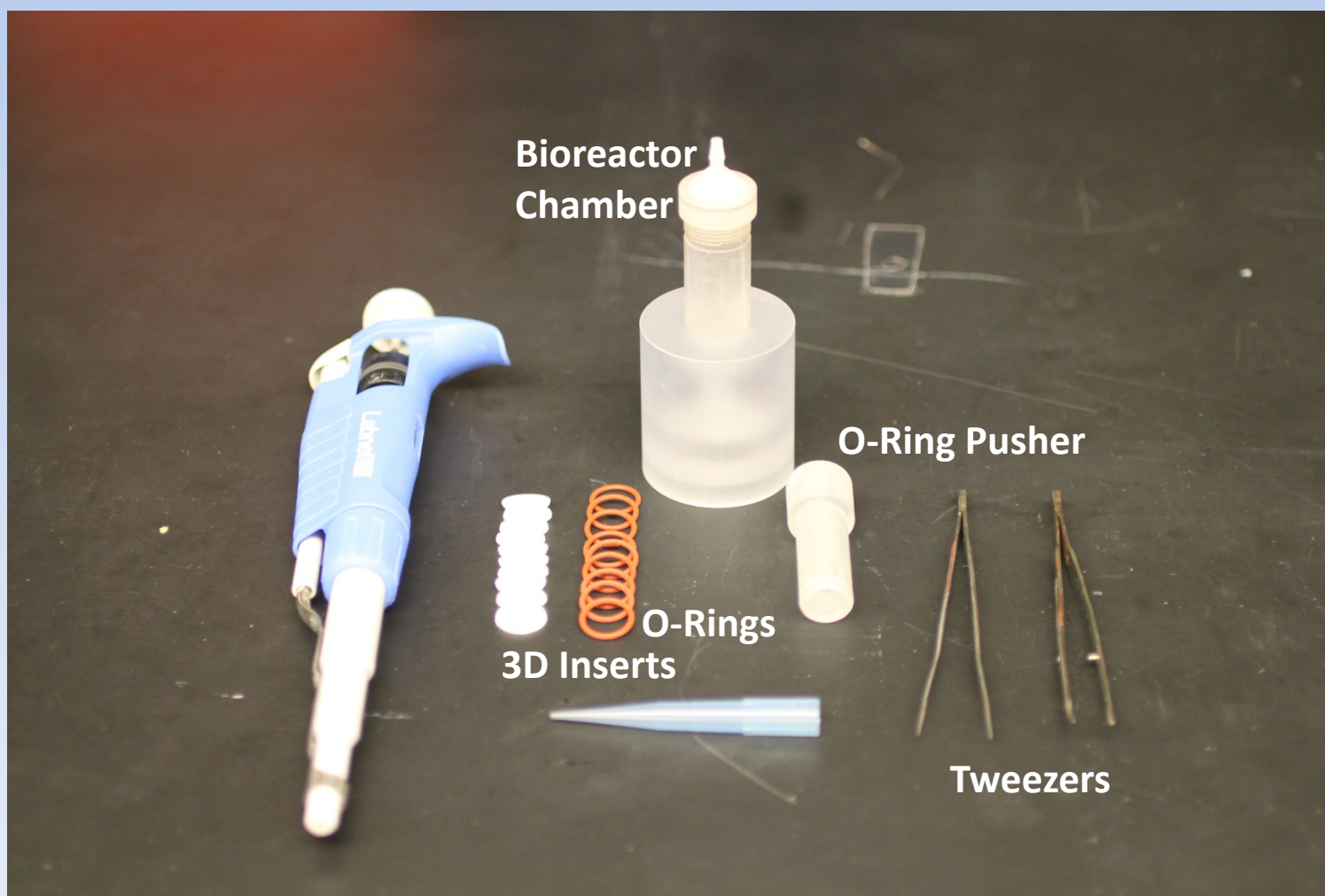


Bioreactor Loading and 3D Perfusion





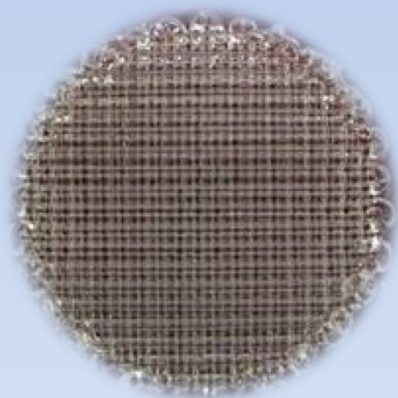
Bioreactor Loading: Parts





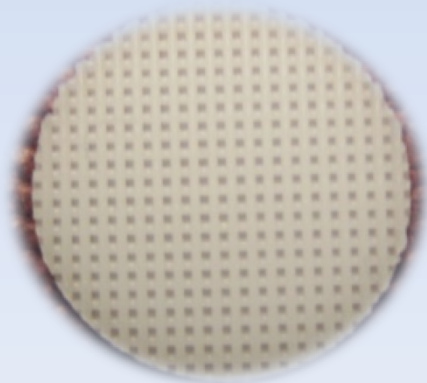
3D Insert™ Series

3D Insert™-Polystyrene



Polystyrene (PS) is a transparent polymer used in traditional cell culture.

3D Insert™-Poly-ε-Caprolactone

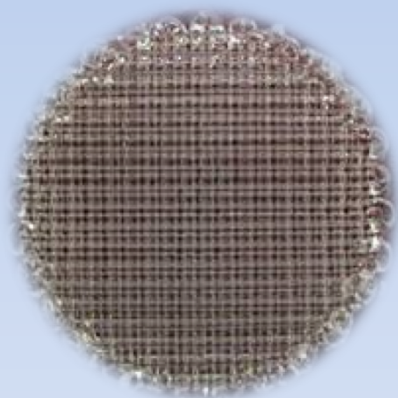


Poly-ε-Caprolactone (PCL) is a biodegradable polymer used in FDA approved medical devices



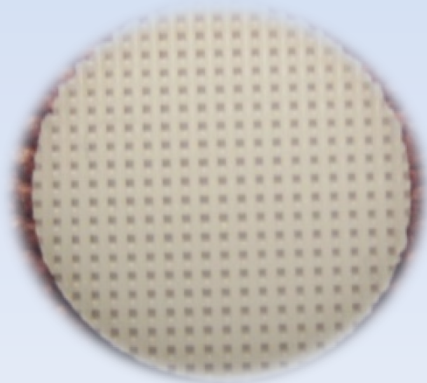
3D Insert™ Series

3D Insert™-Polystyrene



- Configurations: PS(1520) and PS(3040)
- PS(1520) = 150-um Fiber/200-um Spacing
- PS(3040) = 300-um Fiber/400-um Spacing
- Total number of layers: 4

3D Insert™-Poly-ε-Caprolactone



- Configurations: PCL(3030) and PS(3050)
- PCL(3030) = 300-um Fiber/300-um Spacing
- PCL(3050) = 300-um Fiber/500-um Spacing
- Total number of layers: 6



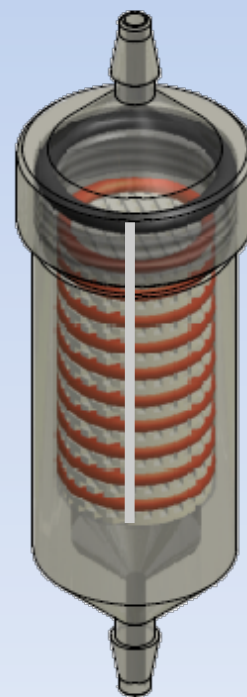
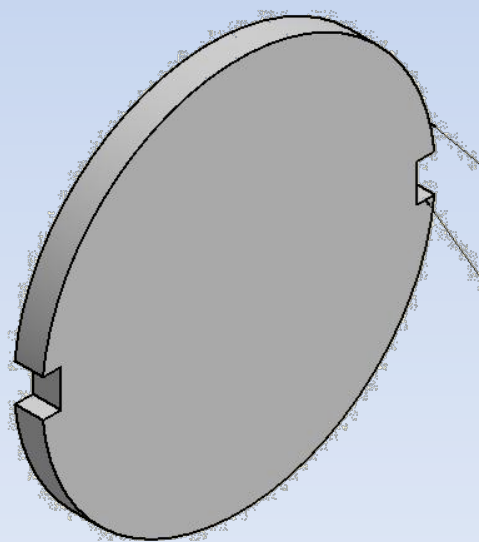
3D Insert™: *Exact Seeding volumes*

Insert™ Format	Seeding Volume (ul)			
	PS(1520)	PS(3040)	PS(3030)	PS(3050)
24-well	60	120	130	160
12-well	120	240	270	340
6-well	300	600	690	880



3D Perfusion: *Specific Features*

Easy-Handle™ 3D Insert



Lateral micro-channels

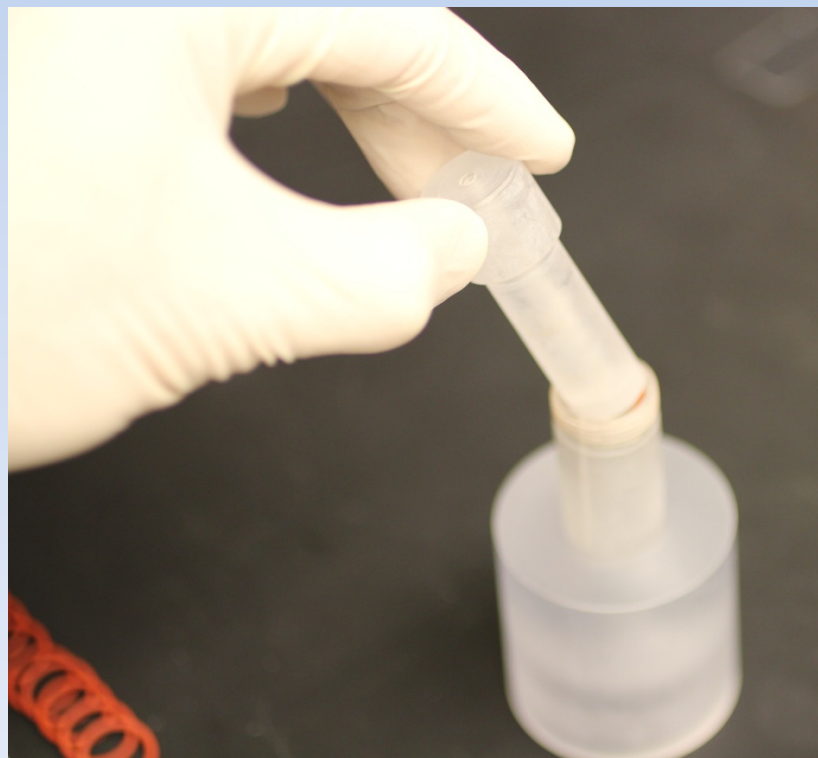


3D Perfusion Seeding: *Step 1*

O-Ring Placement



Pushing O-Ring



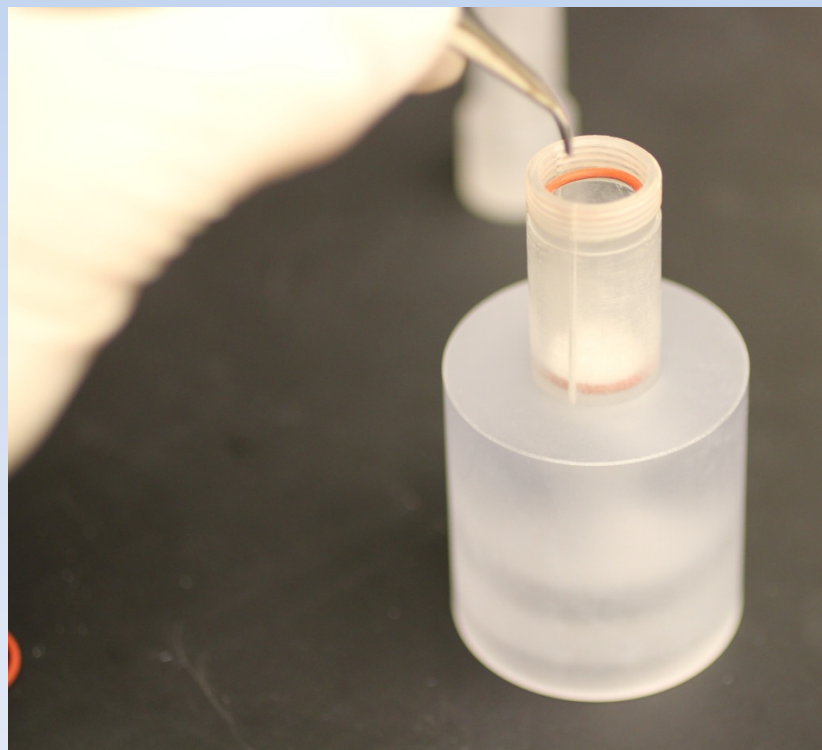


3D Perfusion Seeding: *Step 2*

3D Insert Placement



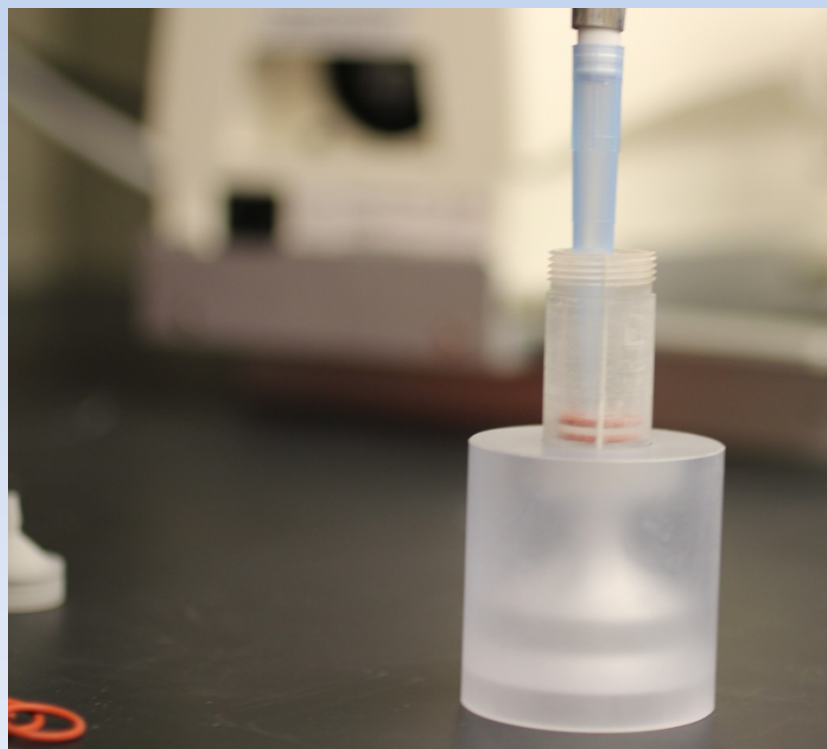
O-Ring Placement



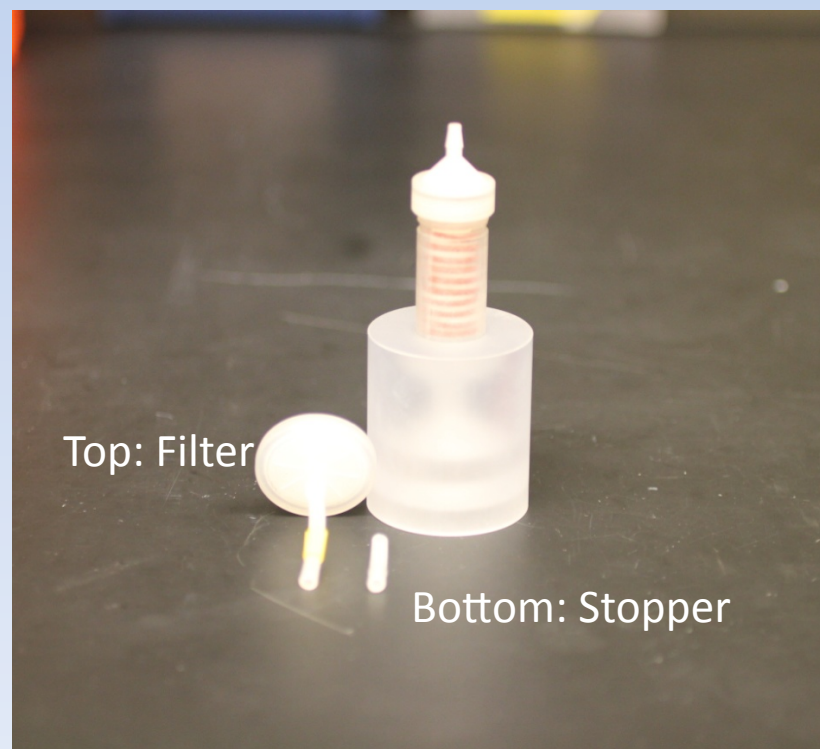


3D Perfusion Seeding: *Step 3*

Cell Suspension Seeding

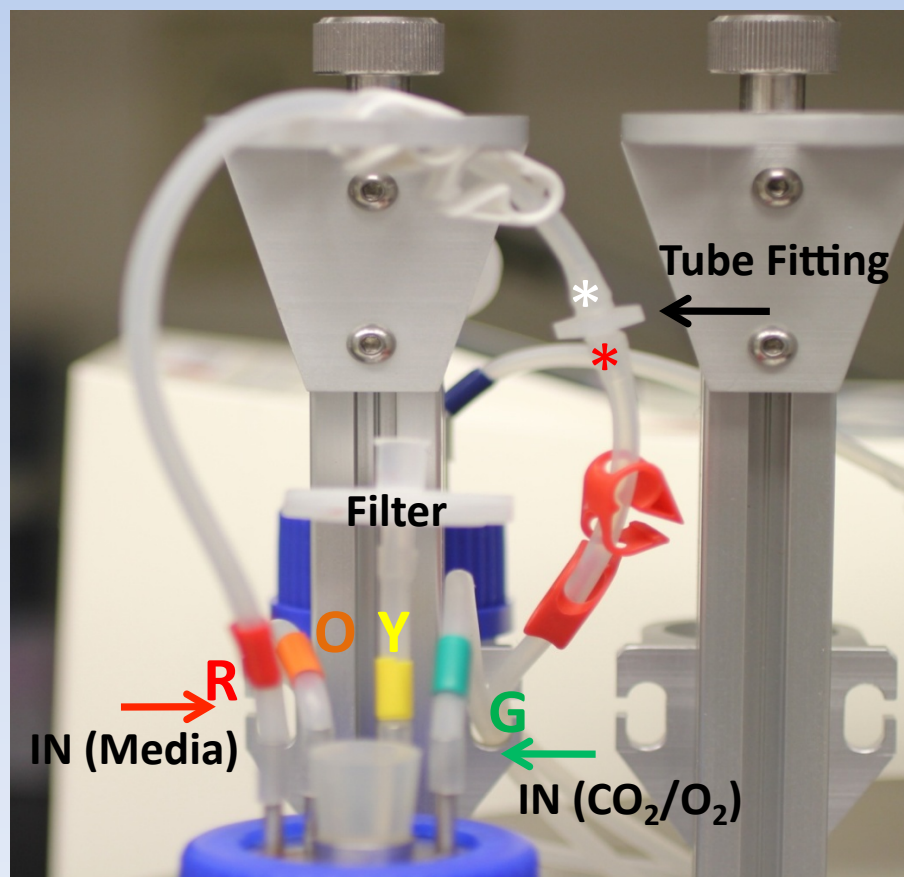


Fully Loaded Chamber

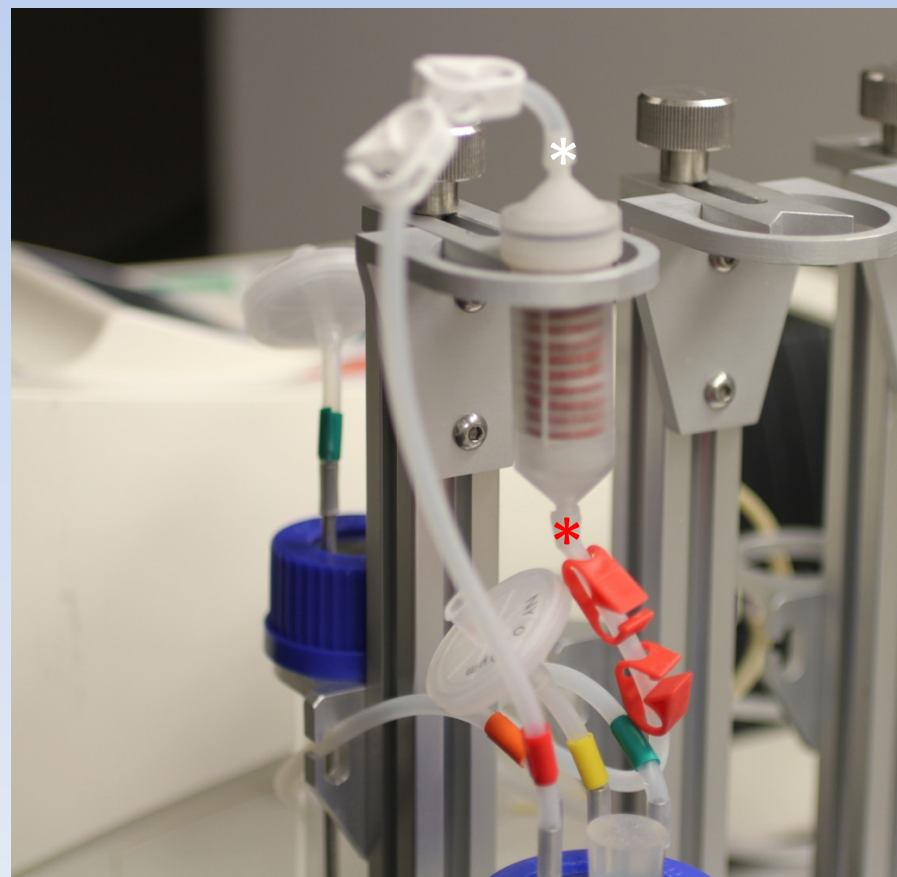




Connecting the 3D Bioreactor Chamber



Media Equilibration



Bioreactor Perfusion



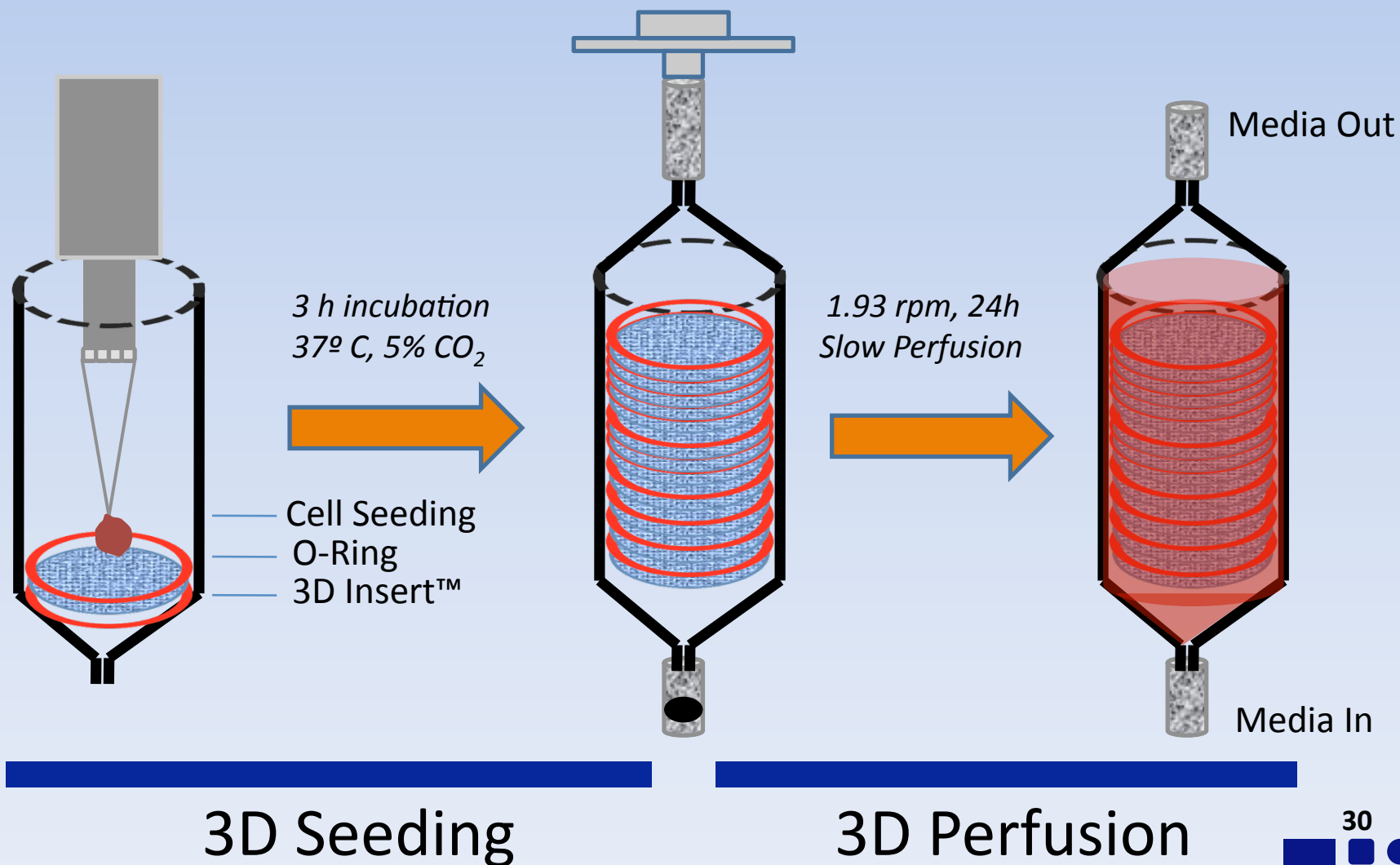
3D Perfusion: *rpm and Velocity*

	Bioreactor Chamber Format		
	24-Well	12-Well	6-Well
Velocity(cm/min)	rpm	rpm	rpm
0.0	0	0	0
0.2	1.93	4.84	12.08
0.4	3.86	9.69	24.15
0.6	5.8	14.53	36.23
0.8	7.73	19.38	48.31

**Pump maximum rpm capacity is 60 rpm (Manufacturer Specifications)*



3D Seeding/Perfusion Protocol



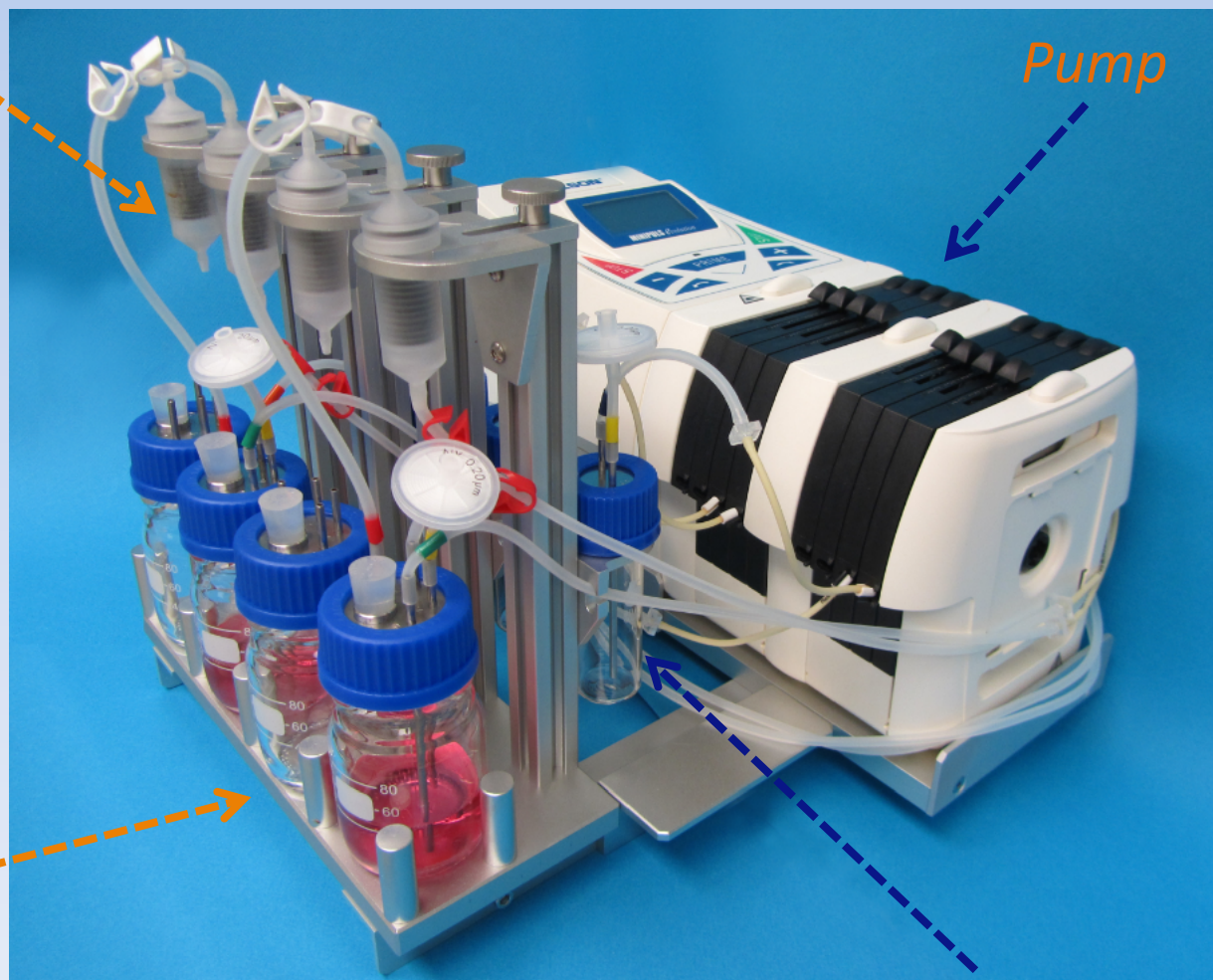


3D Perfusion: *Complete Assembly*

3D Chambers

Pump

Media
Reservoir



CO₂/Humidifier



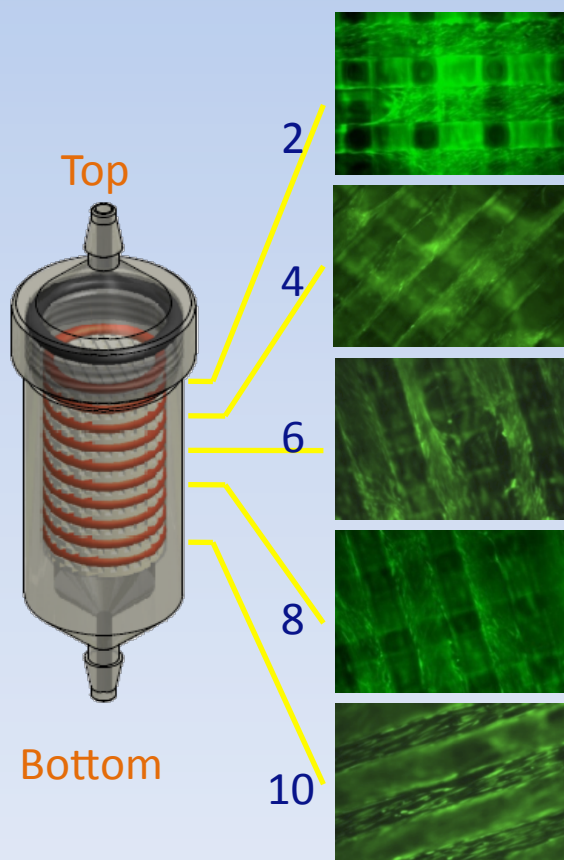


3D Perfusion Bioreactor Validation

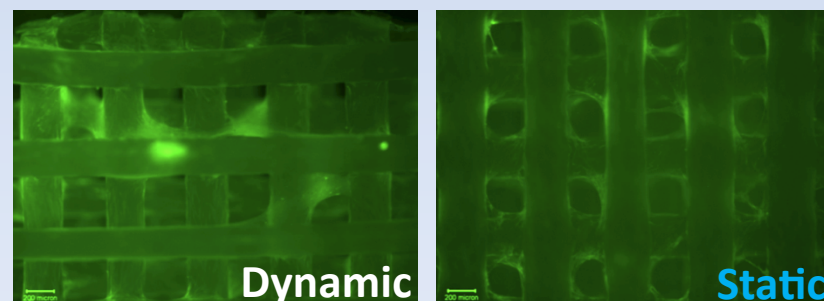
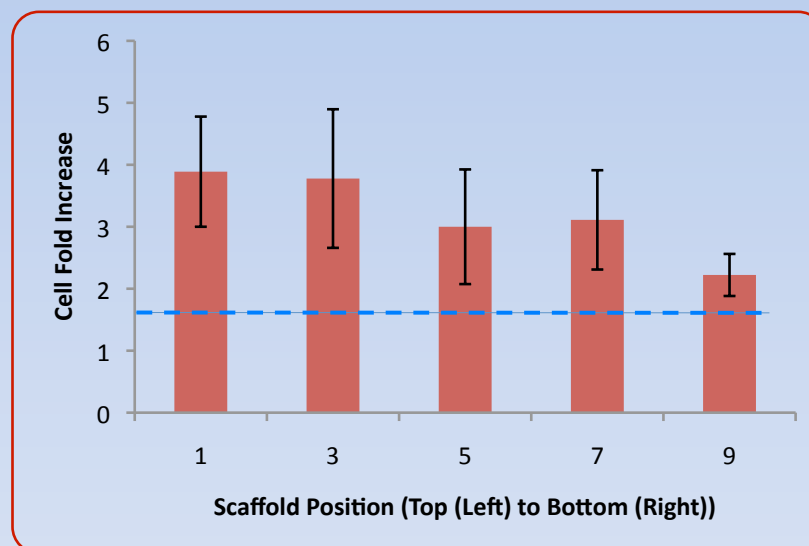




3D Perfusion: *Validation I*



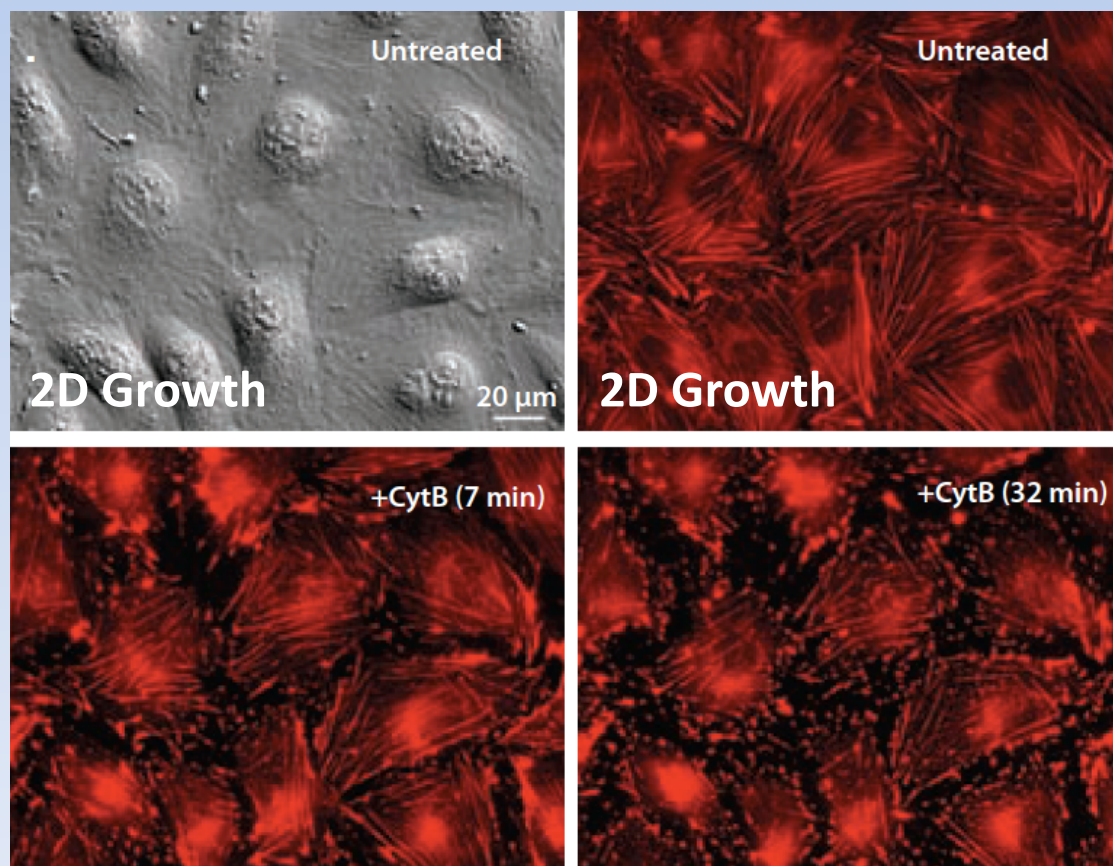
Neonatal Fibroblasts Calcein Viability



Neonatal Fibroblasts Growth in Perfusion



3D Perfusion: *Validation II*



Reporter Cancer Cell Line: ACTbCompoZr™ ZFN: U2OS-β Actin-RFP

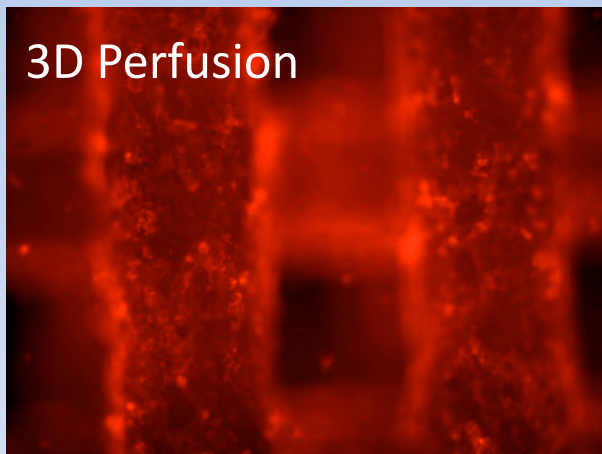




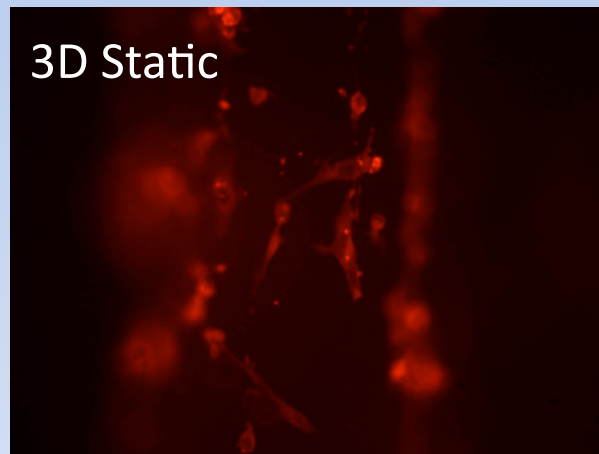
3D Perfusion: *U2OS* Growth

Day 5

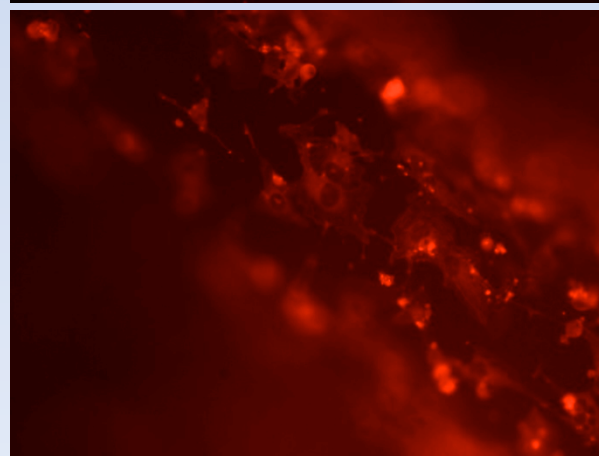
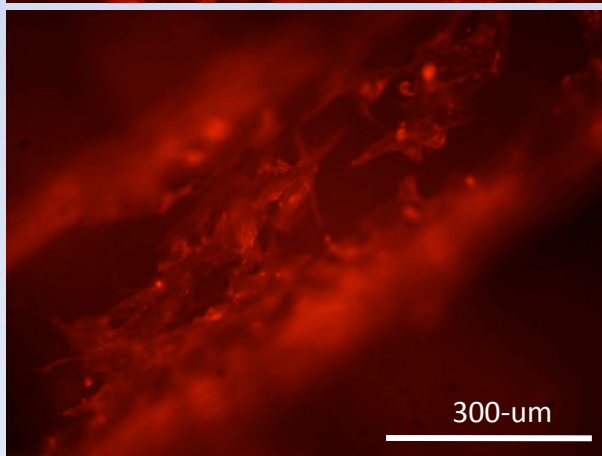
3D Perfusion



3D Static



Day 10



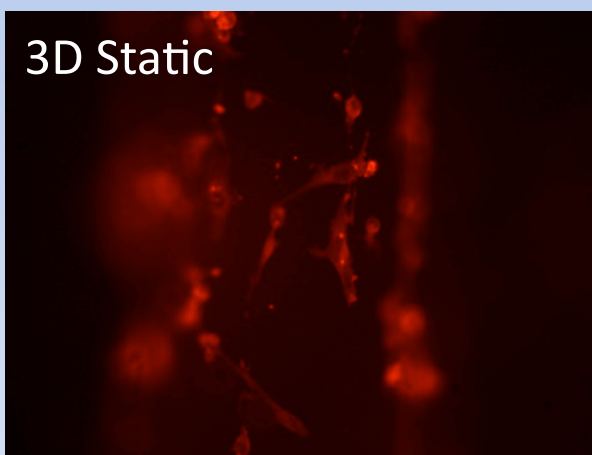
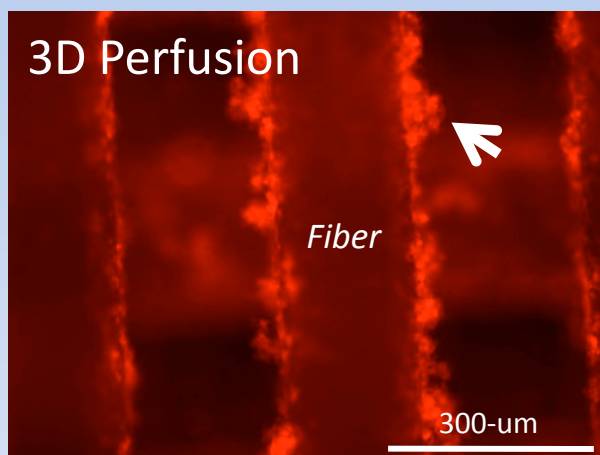
3D Perfusion Enhances Osteosarcoma Growth on 3D Inserts™

35

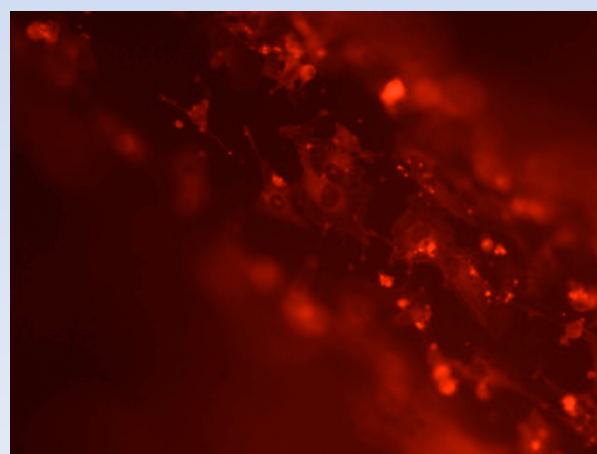
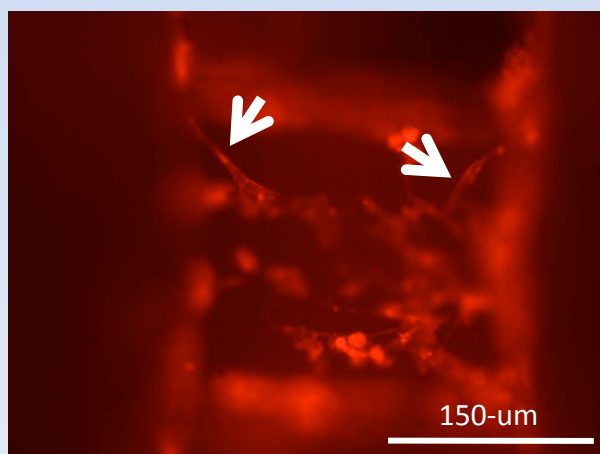


3D Perfusion: *U2OS Cell Morphology*

Day 5



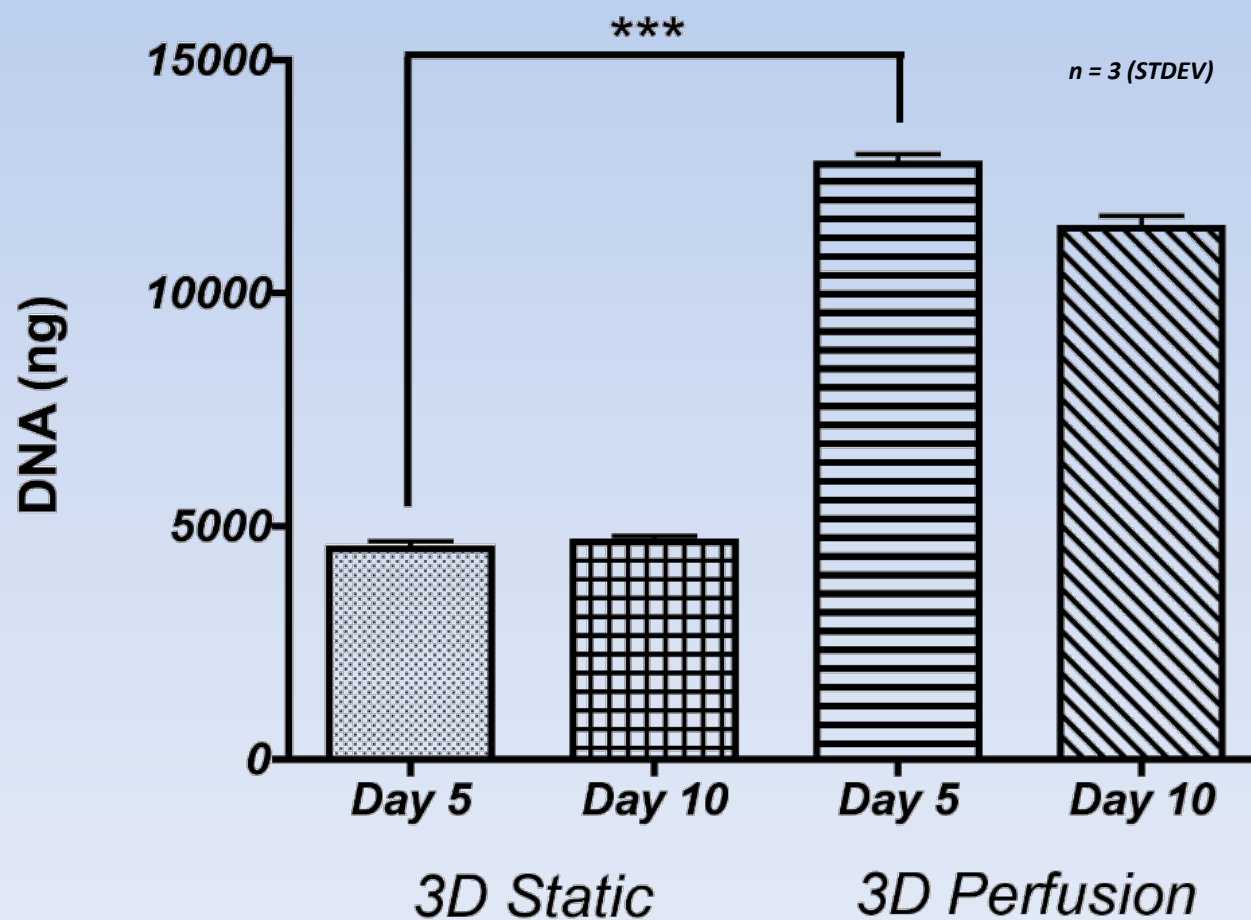
Day 10



3D Perfusion Causes Osteosarcoma Morphology Changes



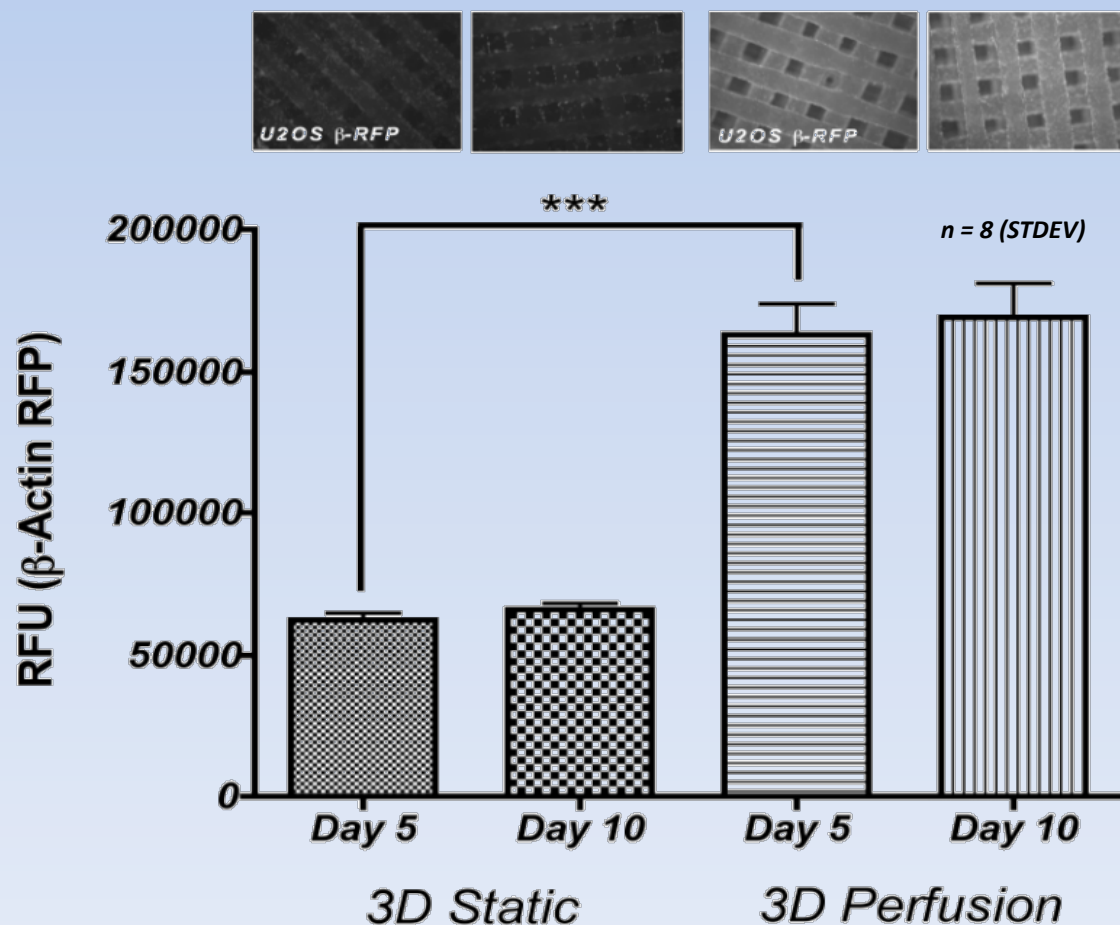
3D Perfusion: *U2OS Proliferation*



3D Perfusion Causes Early Proliferation of Osteosarcoma



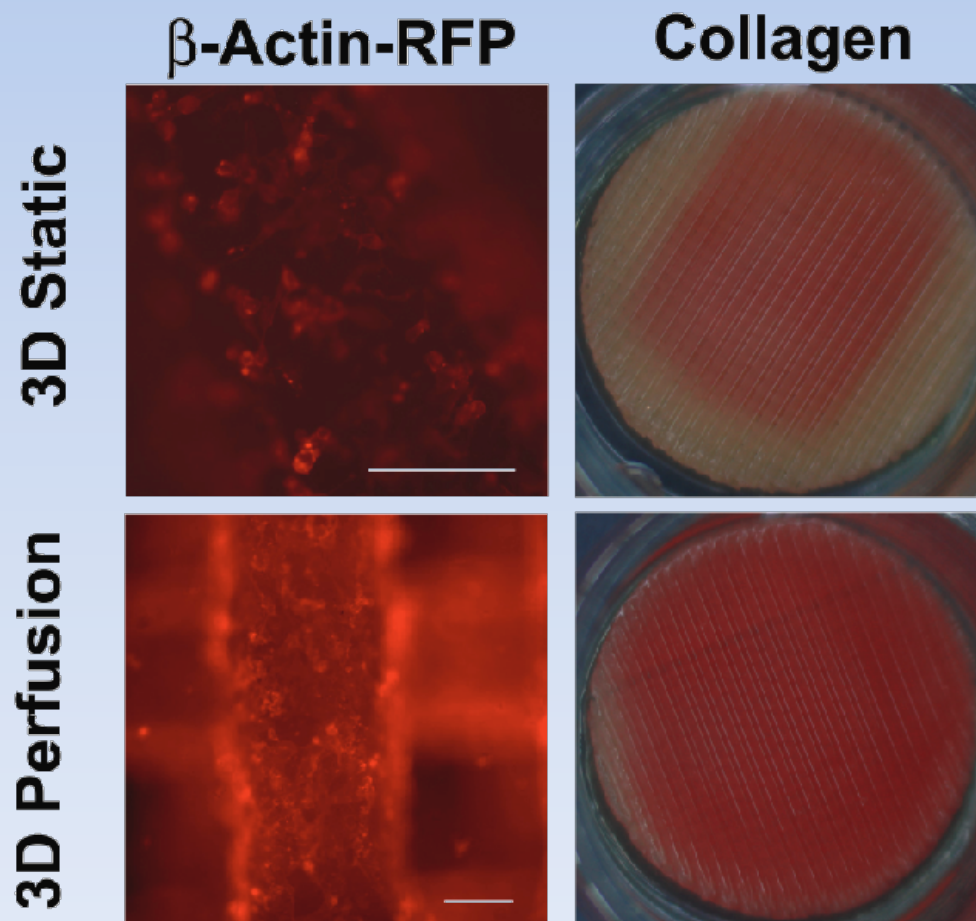
3D Perfusion: β -Actin RFP Expression



3D Perfusion Enhances Beta-Actin Upregulation



3D Perfusion: *Collagenous Matrix*



Total Collagen Staining Shows Increasing Deposition of ECM



Carlos E. Caicedo-Carvajal, Ph.D.
ccaicedo@3DBiotech.com

