Circulatory System and Integrated Muscle Tissue for Drug and Tissue Toxicity

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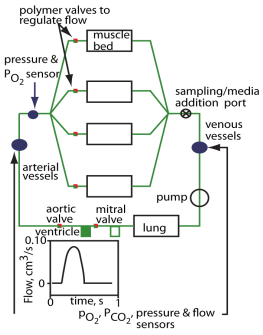
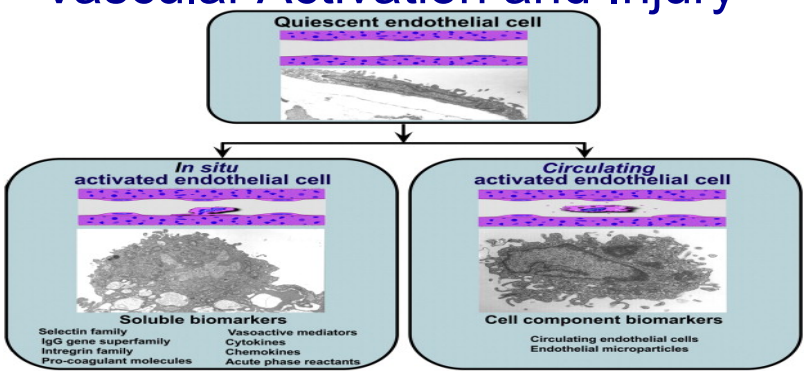
Goal: Develop a circulatory system that consists of a high-pressure arterial system interfaced with 3D skeletal muscle microcirculatory test beds



Rationale- Skeletal Muscle

- Accounts for 40% of the body's mass and plays critical role in metabolism and energy expenditure.
  - Under resting conditions accounts for 20% of cardiac output
  - During during heavy exercise muscle blood flow rises 2-5X
- Major diseases involve skeletal muscle or skeletal muscle metabolism
  - Metabolic disease and diabetes
  - Major tissue involved in insulin-mediated glucose uptake
  - Sarcopenia, age-associated loss of skeletal muscle mass, leads to lower metabolic adaptation and slowed immunological responses to disease
  - Muscular dystrophy
  - Muscle atrophy during cancer treatment
- Drugs know to cause mitochondrial toxicity affect muscle function, such as cerivastatin and thiazolidinediones, which is used to treat type 2 diabetes

Vascular Activation and Injury



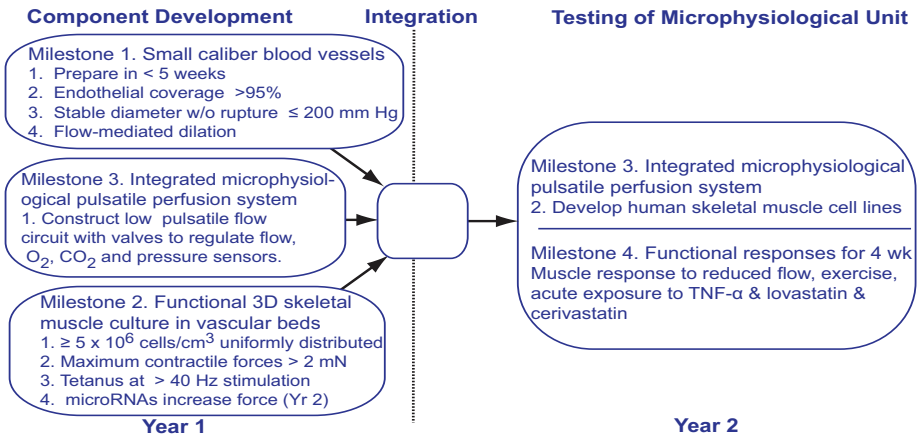
- Endothelial Dysfunction – influences atherosclerosis, sepsis, edema
- Vascular Activation and Injury - autoimmune diseases
- "Drug-induced vascular injury (DIVI) is a nonclinical finding that often confounds the toxicological evaluation of investigational drugs." Toxicol Pathol 2010;38:856-871

Vascular Pharmacology, 56, 2012 14 – 25; http://dx.doi.org/10.1016/j.vph.2011.09.002

Endothelial Cell-Skeletal Muscle Interactions

- Vasodilator release affects muscle function
  - Nitric oxide promotes muscle fiber maturation
- Nitric oxide levels regulate muscle blood flow
- Endothelial dysfunction affects muscle blood flow and function
- Skeletal muscle response to exercise influences angiogenesis
- Endothelial cells affect satellite cell function (*Mol. Biol. Cell (2007) 18: 1397*)
  - In vivo satellite cells adjacent to capillaries
  - Changes in capillary density affect number of satellite cells
  - Myoblast proliferation greater when cocultured with ECs

OVERVIEW



Primary Human Cell Sources

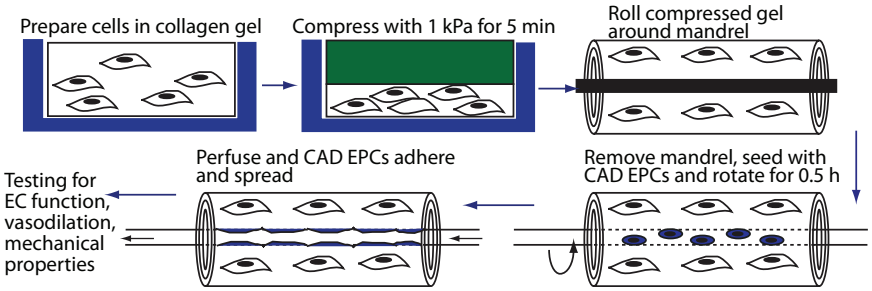
- **Endothelial cells** - derived from endothelial progenitor cells isolated from adult and umbilical cord blood
- **Fibroblasts** - neonatal and adult dermal fibroblasts (ATCC and Invitrogen)
- **Skeletal muscle myoblasts** - biopsies from the *vastus lateralis* of healthy middle-aged volunteers
- iPSC –capture range of variability

Functional Requirements for Microphysiological Blood Vessels and Muscle

- Enable validation and qualification of microphysiological system
- Small Diameter Arteries
  - Flow mediated vasodilation – in vivo 6-8% change in diameter for physiological shear stresses
- 3D Engineered Human Skeletal Muscle
  - Production of contractile forces – specific force between 150-320 kN/m^2 in soleus and tibialis anterior
  - Oxygen consumption
- Complement with metabolomic measurements
  - Nondestructive and end-point assays
  - Database of human responses in vivo and in vitro

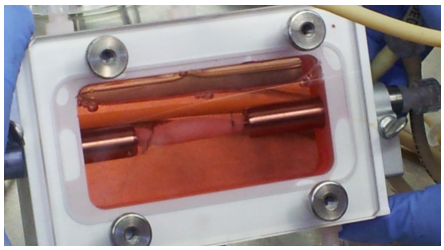
MILESTONE 1 Small Caliber Blood Vessels

TEBV Generation



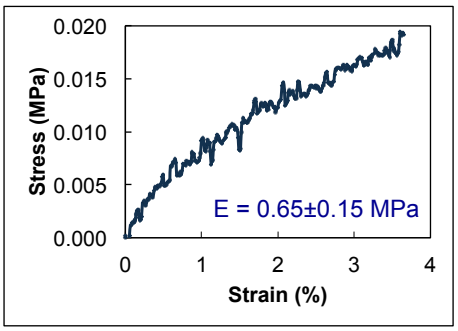
Adapted from: Biomaterials, 2013. 34: 1954-1966 ;
Methods in Molecular Biology: 3D Cell Culture: Methods and Protocols, 2011:183-196.

Vessel Constructs from Dense Collagen Sheets



Vessel may be endothelialized and perfused immediately after rolling

TEBV perfused for 7 days at 1 mL/min.



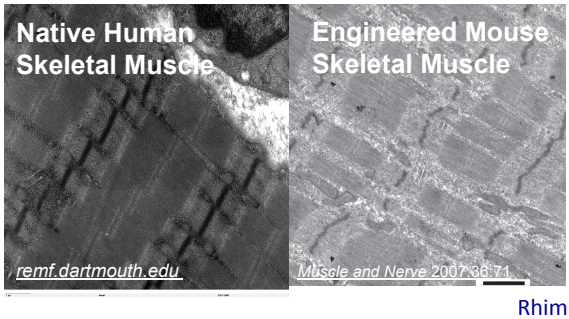
Burst Pressure exceeds minimum specification

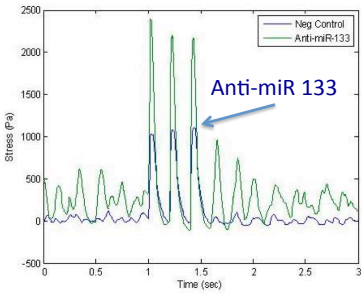
<u>TEBV generation time</u>
Dense collagen sheets : 2 weeks
Cell sheet engineering: ≥5 weeks

Next Steps to Validate

- Optimize endothelialization conditions
- Flow mediated vasodilation
  - Testing with and without endothelium
  - Demonstrate specificity with inhibitors of endothelial nitric oxide synthase
- Stability of response over 4 weeks
- Decrease diameter from 1000 μm to 750-800 μm

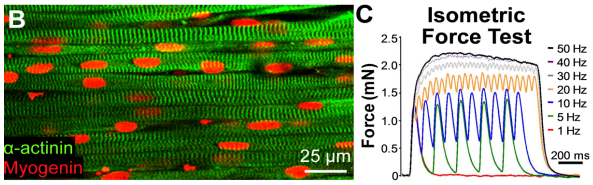
3D Skeletal Muscle





Rhim et al. 2010. Tissue Eng Pt A 16:3589

Alignment increases force production (Bursac)

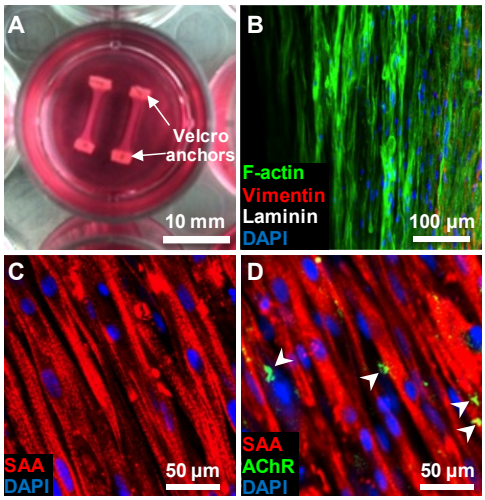


Bian et al. 2012. Tissue Engineering A 18:957-67

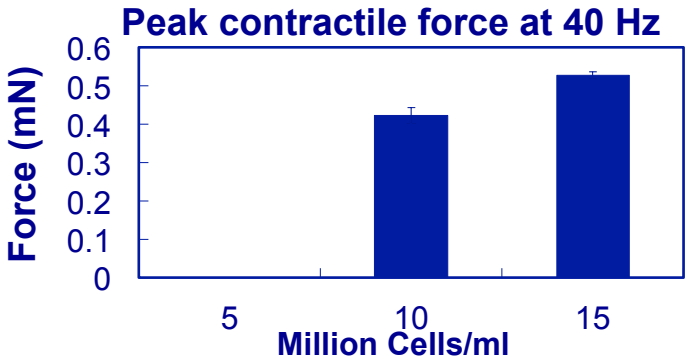
Milestone 2: 3D Human Skeletal Muscle

- Goals and timeline:
  - 2.1. Utilize hydrogel-based approach to examine two designs to produce highly aligned and differentiated human skeletal muscle with an endothelialized surface.
  - 2.2. Use microRNAs and/or mechanical stimulation to promote differentiation of muscle tissue
- Criteria for success:
  - 1. Construct contains at least 5 x 10^6 cells/cm^3 with muscle fibers distributed uniformly.
  - 2. Muscle produces maximum contractile forces > 2 mN when electrically stimulated and exhibits tetanus when stimulated at frequencies ≥ 40 Hz.

Developed stable 3D human skeletal muscle bundles and decreased volume by 4X



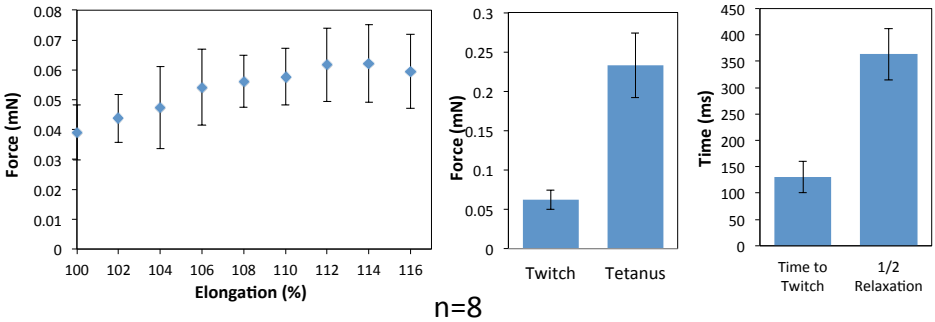
Two Week Cultures



Tetanus: Twitch	**2.4 ± 0.9**
P_0/CSA	**5.5 ± 1.3 kN/m^2**

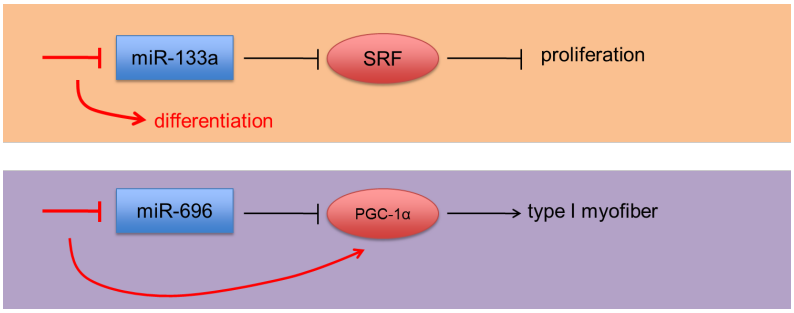
Human Skeletal Muscle Bundles are Functional at 4 Weeks

- Starling-like force-length relationship
- Increased force with tetanic stimuli

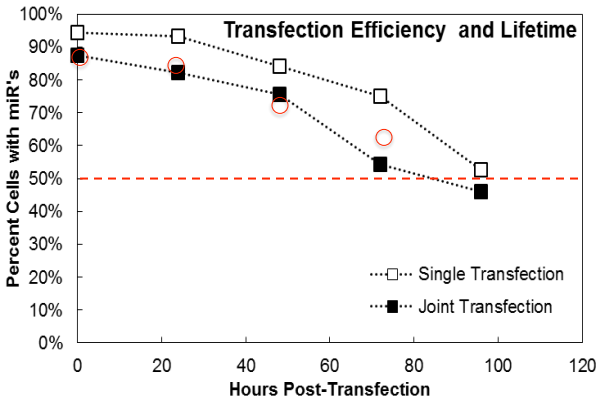


n=8

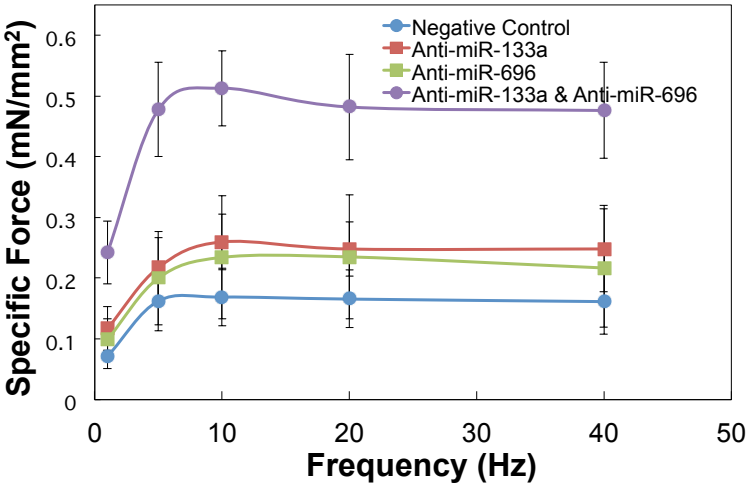
Transient transfection of microRNA's in muscle bundles



Long microRNA retention within human skeletal myoblasts



Proof of principle:
Joint miR-133a/miR-696 inhibition increase specific force



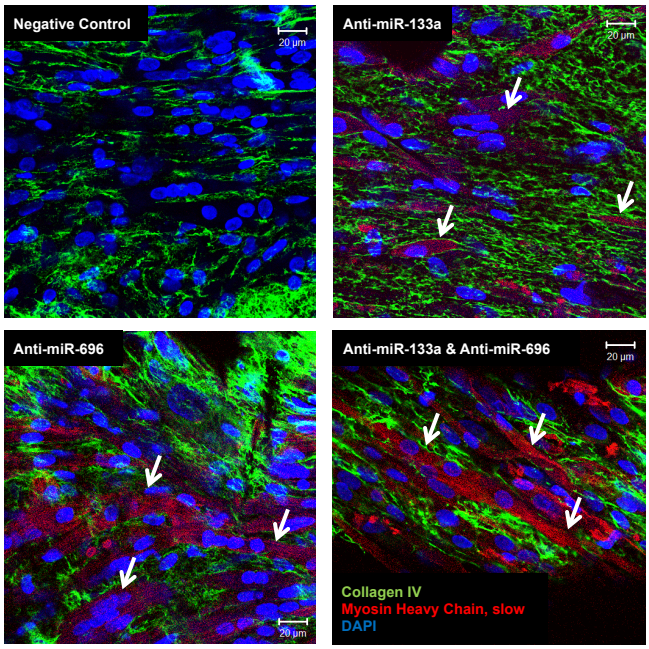
MiR-696 inhibition increases presence of type I myofibers

Little/no type I myofibers

Few, short type I myofibers

Longer, misaligned type I myofibers

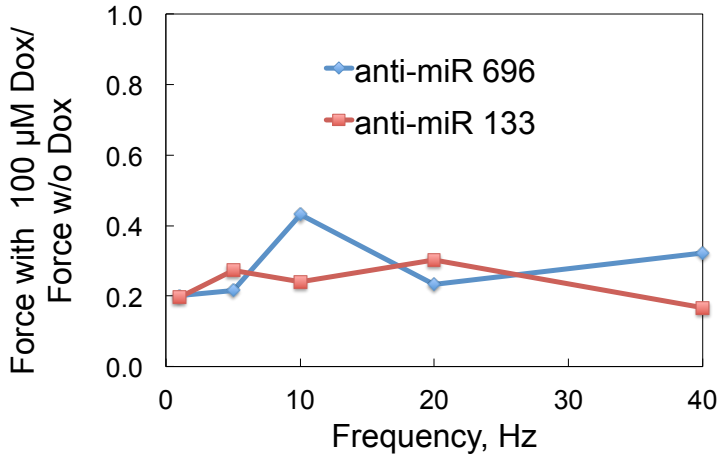
Longer, aligned type I myofibers



Candidates for Toxicity Testing

Drug	Key Function	Effects Upon Skeletal Muscle
Lovastatin	Statin to lower cholesterol	Limited mitochondrial toxicity
Cerivastatin	Statin to lower cholesterol	Mitochondrial toxicity, reduced fatty acid metabolism
Taxol	Inhibits cell proliferation	Microtubule stabilizing agent, reduced proliferation, loss of endothelium, apoptosis
Antimycin	Piscicide; inhibits ubiquinol:cytochrome C oxidoreductase	Mitochondrial toxicity
Rotenone	Pesticide; inhibitor of NADH dehydrogenase	Mitochondrial toxicity
Doxorubicin	Anti-tumor agent; interacts with DNA and halts replication	Fatigue and reduced exercise capacity

Doxorubicin Treatment Significantly Reduces Contractile Force

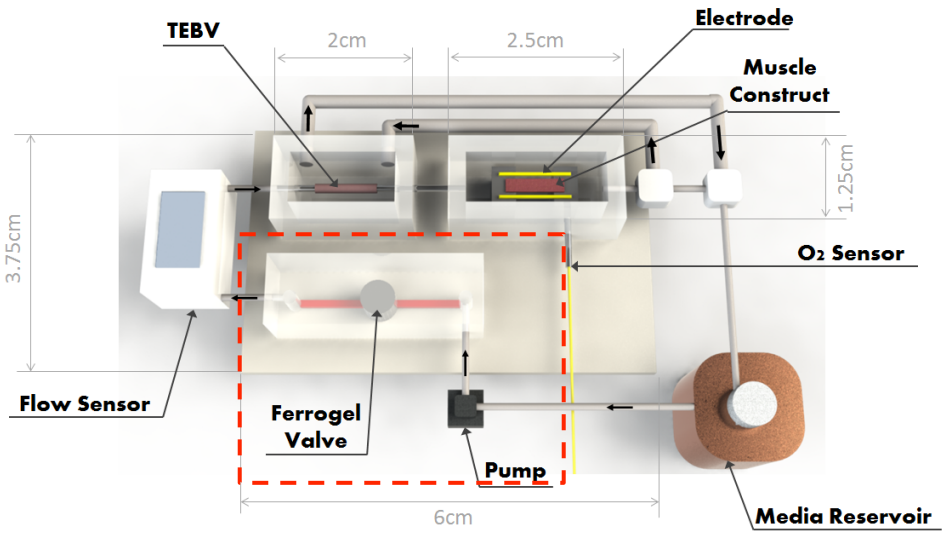


- Exhibits greater sensitivity than primary mouse muscle fibers (British Journal of Cancer (2009) 100, 311)

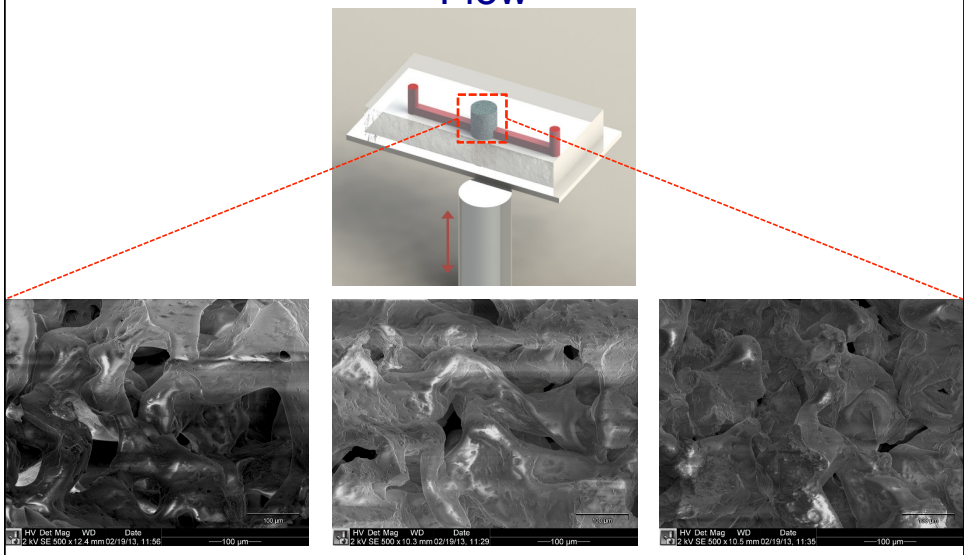
Ongoing/Next Steps

- Incorporation of muscle bundles into the flow loop
- Endothelialization of muscle bundles
- Optimize force production
- Dose response studies
- Construction of microperfusion system for long-term studies

Microphysiological Pulsatile Perfusion System



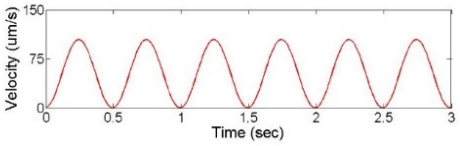
Magnetically-Active Polymer Sponge to Regulate Flow



7mm apart from magnet | 3.5mm apart from magnet | Fully contact to magnet

Validation of Pump

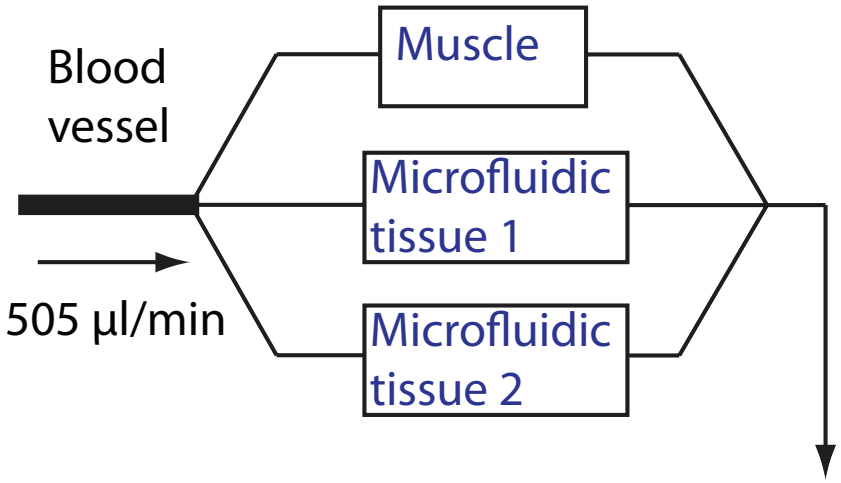
- Frequency response – 1-10 Hz
- Flow Range – Reproducible over range 1-10 μL/min
- Can work with tissue culture media
- Response reproducible for 3 days – long term studies in progress
- Biocompatible



Milestone 4. Evaluate functional responses (Year 2)

1. Establish that pulsatile perfusion system with blood vessels and muscle tissue is stable and evaluate response of muscle tissue to 20% to 70% reduction in flow rate and acute simulated exercise.
2. Establish response of blood vessels and muscle to acute exposure to TNFα.
3. Establish response of muscle tissue to lovastatin and cerivastatin.

Scaling Flow Conditions and Predicting Drug Distribution



Flow in Microphysiological System Compatible with Microfluidic Units

Organ	Percent of Body Mass	Percent of Cardiac Output	Flow in Parallel Microfluidic & Microphysiological Units, 200- 505 µl/min	Cells x 10^5
Brain	2 %	13	28-66 µl/min	0.21 - 0.32
Skeletal muscle	42 %	21	400-106 µl/min	4.5 - 6.75
Heart	0.4%	4	8-20 µl/min	0.05 - 0.07
GI Tract	4.0%	24	45-120 µl/min	0.44 - 0.66
Kidney	0.5%	19	38-96 µl/min	0.05 - 0.07
Lung	0.4%		200-505 µl/min	0.04 – 0.06
All other organs	51.1%	19	45-96 µl/min	

- Physiologically-based
  - Correct ratio of organ flow rates, masses, fluid volumes and organ residence times

Summary

- Functional assays represent a useful approach to provide validation of microphysiological systems
- Supplement with metabolic, molecular and histological analysis
- Developing testbed for physiological perturbations
- Issues being addressed –
  - Ratio of fluid, cell and matrix volumes
  - Vessel size
  - Cultrue media composition
  - Materials to minimize

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