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Research Description Summary
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Background: The grand challenge of our work is to engineer integrated biological machines, or "*bio-bots*", which are clusters of living cells that have desired functionalities and can perform prescribed tasks. We have focused our initial efforts on demonstrating simple locomotion using muscle cells. Physical and directed movement of an object requires asymmetric actuation of sub-components of that object. The basic building block for the locomotion function in our bio-bot is a novel 'biological bimorph' cantilever structure that actuates in one direction.

Fabrication and SLA: We fabricate these cantilevers with a 3D stereolithographic printer, a layer-by-layer rapid prototyping system. The 3D stereo-lithographic printing apparatus uses a high wavelength UV laser (325nm) to directly write on the surface of a photosensitive material by an additive layer-by-layer method. The pattern is defined in the computer through 3D reconstruction of CAD software. A mixture consisting of poly(ethylene glycol) diacrylate (PEGDA) and acrylic-PEG-collagen (PC) was formulated as the photopolymerizable material for fabricating cantilever beams. Collagen I from rat tail was modified with acrylic groups to UV cross-link to the PEG backbone in the presence of a photoinitiator. The cantilever beams can be tuned to a wide range properties.

The design is promising for the future of cell-based bio-hybrid actuators, which can have implications in drug delivery, health, and the environment. In the future, these biological machines could be controlled by light, chemical cues, or other factors, in conjunction with various cell types such as neurons and endothelial cells, to create an entire system.

Optogenetic Control: Optogenetic C2C12s (mouse skeletal muscle cells) are transfected with a gene to express channelrhodopsin-2, a membrane ion channel that responds to light. When the cells are subjected to blue light, contraction can occur. This allows for another level of control for skeletal muscle cells, which do not contract spontaneously as cardiac cells do. Optogenetics also eliminates the need for electrical stimulation.

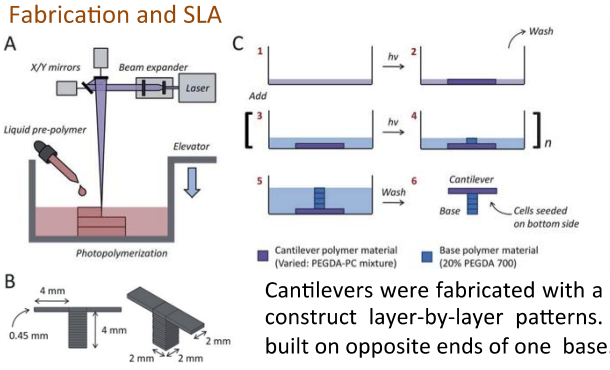
Future steps: We have been quantifying muscle cell differentiation based on culture conditions and the presence of insulin growth factor (IGF) using immunohistochemistry markers that will demonstrate cell maturation and fusion. The next steps for the project include gathering data on the bending angles, displacement, and surface stresses on the cantilevers with optogenetic C2C12s to examine how we can harness the maximum cellular contraction force. Alignment of the cells with a micro-contact printing technique may also lead to greater contraction force and movement.

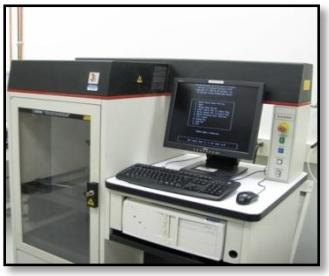
microSLA: A projection stereolithography system is being developed that will allow us to pattern structures on a physiologically relevant scale with a feature size of 10-20μm. The setup makes use of a digital micro-mirror device, or DMD, as as a digital mask to polymerize the material with 365nm UV light based on user-specified geometry. Plans for the near future include measurement of the resolution and minimum feature sizes, characterization of the energy dosage and cure depths based on material properties, demonstration of patterning, and cell encapsulation and viability studies.

References:

Chan, V., et al. (2012). Multi-material bio-fabrication of hydrogel cantilevers and actuators with stereolithography. *Lab on a Chip*, 12, 88–98.

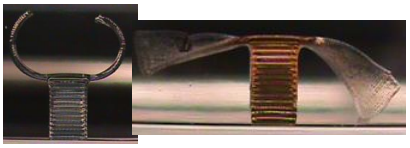
Fabrication and SLA





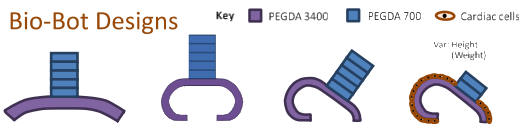
Cantilevers were fabricated with a 3D SLA, which uses a UV laser to construct layer-by-layer patterns. Two separate cantilevers were built on opposite ends of one base.

We varied the thickness of the cantilevers, the length of one cantilever, and the height of the body. Asymmetry maximizes motion in one direction. All of these parameters can be used to control the contact area of the cantilever with the surface.

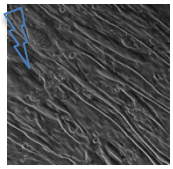


A sheet of cells on the back side of the cantilever creates tension that curls the cantilever down to make contact with the surface. When the sheet actuates, the cantilever bends forward, causing a build up of stress that is released in the form of motion when the sheet relaxes.

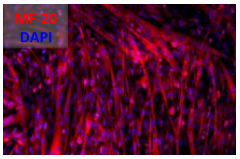
Bio-Bot Designs



Optogenetics



C2C12s (mouse skeletal muscle cells) are transfected with a gene to express ChR-2, a membrane ion channel that responds to light. When the cells are subjected to blue light, contraction can occur.



Immunohistochemistry

We have used insulin growth factor as a marker to demonstrate C2C12 differentiation.