Synthesis of PDMS with Fluorescent Beads for Analysis of Muscle Cell Contraction

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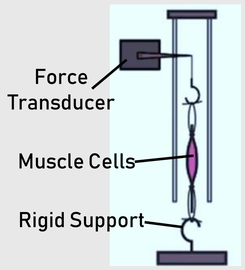
Background and Project Overview

- Cultured muscle cells are a valuable model for *in vitro* drug screening and toxicity studies.

Drug/Chemical → Cultured Muscle Cells → Muscle Cell Contractions

- The current method used to analyze the force of cultured muscle cell contractions involves the setup shown to the left.[1]
- However, force transducers are expensive, making high-throughput screenings using cultured muscle cells impractical, as a large number of transducers are required.



- The goal of this project was to produce a durable, flexible, and biocompatible polydimethylsiloxane (PDMS) film with embedded fluorescent beads that can be attached at one end to cultured muscle cells and at the other end to a rigid support.
- As the cells are stimulated and contract, the PDMS film would be stretched, causing the fluorescent beads to move.
- This movement could then be tracked and analyzed to gain information about the strength of the muscle cell contractions.

Methods

PDMS Synthesis and Bead Integration

- It was required that the PDMS film be biocompatible, transparent, and durable, with an elastic modulus of ~5 kPa.
- Based on literature data, it was determined that Sylgard 527 in a 1:1 crosslinker to base ratio would fulfill these requirements.[2]
- The following process was used to produce the PDMS films:

Pipet 5 μL of the 0.5 μm-diameter fluorescent beads into a weigh boat and dry in a vacuum overnight

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Use a pestle to grind the beads back into a powder (~5 minutes)

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Add 500 μL of crosslinker and 500 μL of base to the dried beads and mix using a pipet tip for 5 minutes

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Pour mixture into mold constructed as shown in the diagram below

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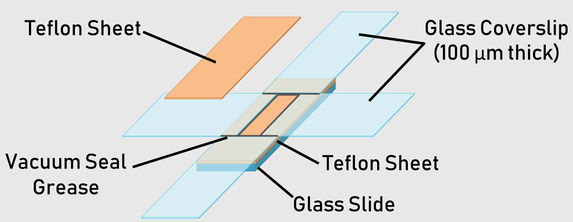
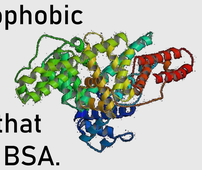
Incubate PDMS mixture overnight at 70 °C to cure the film

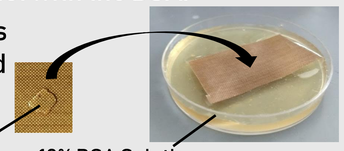


Surface Modification

- Bovine serum albumin (BSA) was used to modify one side of the PDMS film because it is able to adhere to hydrophobic surfaces such as PDMS.[3]

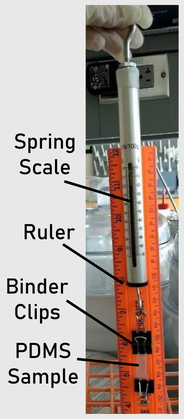


- The Teflon sheet with the attached PDMS film was placed face down in a 10% BSA solution so that only one side of the film was in contact with the BSA.
- After sitting overnight, the film was removed from the solution, rinsed with distilled water, and dried.



Determination of Elastic Modulus

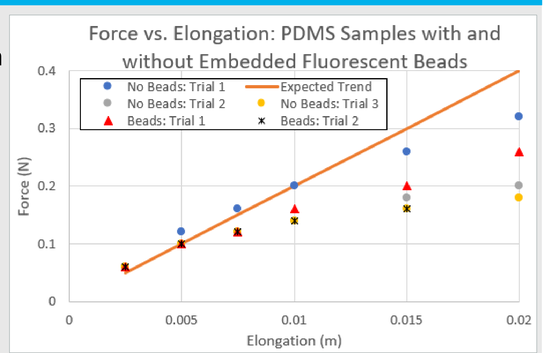
- Samples were made using agarose rather than glass coverslip spacers, giving films with dimensions of 1.5 cm x 4 mm x 1.5 cm.
- Samples were cured at 37 °C for 1 to 4 days before being attached to the spring scale as shown in the image to the left.
- The samples were stretched 0.25 cm, 0.5 cm, 0.75 cm, 1 cm, 1.5 cm, and 2 cm from their resting lengths, and the force read by the scale at each length was recorded



Results

Determination of Elastic Modulus

- A theoretical trendline for a 5 kPa elastic modulus was generated using the equation $F=E*A*(l/l_o)$ where E is 5 kPa, A is 0.6 cm^2, l is the stretched length, and l_o is the unstretched sample length



Plot of theoretical and experimental forces and elongations for PDMS samples with and without beads

- The experimental values for force at each elongation were plotted against this theoretical line.
- Experimental values followed the trend line for elongations below 5 mm, with deviations occurring beyond this point.
- Trial 1 with no beads had a longer incubation time, resulting in a stronger sample (greater force required for elongation).
- The trials with beads displayed similar values to those without.
- If elongation stays below 5 mm, PDMS samples with and without beads display the required elastic modulus of 5 kPa.

PDMS Synthesis and Surface Modification

- The 100 μm thick films were cut to the desired shape using a razor blade and removed from the Teflon with tweezers.

Cured PDMS films on Teflon Sheets

PDMS film removed from Teflon sheet

- When PDMS samples were modified using BSA, surface modification lasted for about one day, likely due to the migration of uncured PDMS oligomers from the bulk to the surface of the film and polymer chain mobility.[3]

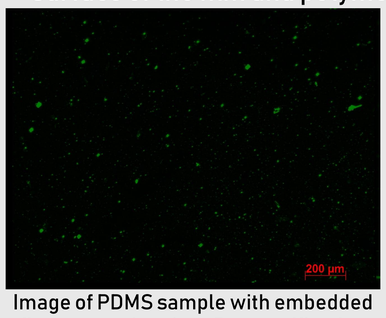


Image of PDMS sample with embedded fluorescent beads at 5x magnification

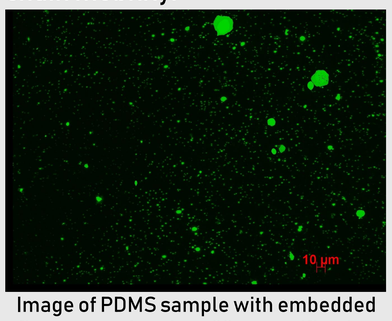


Image of PDMS sample with embedded fluorescent beads at 20x magnification

- Beads were more easily viewed at 20x, and while some areas had beads distributed fairly evenly, other areas had very few beads or large aggregations of beads.

Conclusions and Future Work

- The experimentally determined elastic modulus of 5 kPa should allow muscle cell contractions to stretch the PDMS film easily and evenly, as long as elongation is less than 5 mm.
- The PDMS films synthesized using the Teflon and coverslip mold are of uniform thickness, and surface modification with BSA makes these films temporarily easier to manipulate.
- Alternative, longer-term surface modification techniques may be explored if temporary modification proves insufficient.
- However, the uneven distribution of beads seen in the fluorescent images will make it difficult to effectively track bead movement as the film is stretched.
- Alternatives to these beads, such as microcontact printing of proteins onto the PDMS surface, are being investigated as they may provide a more uniform pattern for tracking movement.[4]

References and Acknowledgements

1. Moorwood, C. et al. (2013). Isometric and Eccentric Force Generation Assessment of Skeletal Muscles Isolated from Murine Models of Muscular Dystrophies. *Journal of Visualized Experiments*: JoVE, (71), 50036.
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4. Yoshie, H. (2016). *Novel Biomechanical Silicone Assays to Quantify Cellular Contractile Forces* (Master's Thesis).

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