

# High-throughput manufacturing of in vitro muscle sheets to test mobility-restoring therapies

## Introduction

Neuromuscular diseases such as muscular dystrophies, sarcopenia, and ALS severely impair mobility and quality of life. Tissue engineering has emerged as an effective strategy to address these challenges. Skeletal muscle cultures provide valuable preclinical models for drug screens and investigations of disease mechanisms, bridging the gap between simple cell culture and animal studies [1]. 3D muscle "rings" between flexible cantilevers are well-established in vitro models of skeletal muscle for applications ranging from disease modeling [2] to biohybrid robotics [3], but their complex fabrication limits scalability and is incompatible with high-content microscopy. In contrast, 2D muscle cultures are scalable and imaging-friendly but suffer from delamination and disorganized contractions, making force measurements inconsistent. This complicates screenings of mobility-restoring drugs, which require careful comparison of forces generated by in vitro muscle tissue replicates. These problems have been solved by the MIT Raman Lab's technology called STAMP (Simple templating of actuators via micro-topological patterning) [4], which utilizes soft substrates and microgrooves to guide muscle fiber alignment (Fig. 1). This strategy, at the interface of 2D and 3D, is referred to as "2.5D".

Building on this innovation, my project focuses on enabling high-throughput muscle contractility readouts by scaling up STAMP to a full 96-well plate of 2.5D muscle cultures. Once optimized, this system will allow the screening of clinically tested compounds to assess its predictive value in pharmaceutical research.

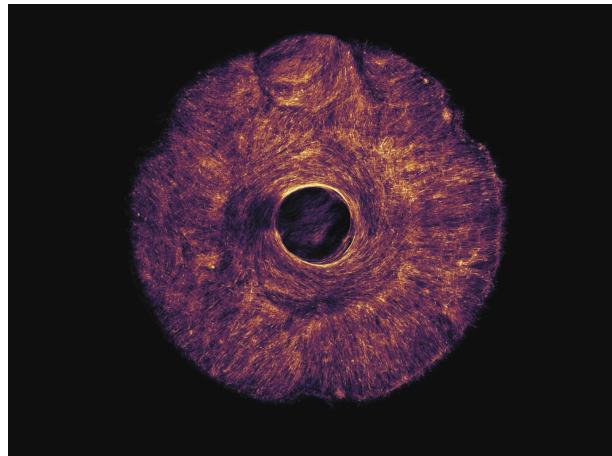


Figure 1: Example of STAMPed muscle tissue with microtopography inspired from iris architecture [4].

## Methods

My early research, ranging from a morphing tri-copter to a music-responsive cello fingerboard, sparked my interest in programmable, adaptive systems, a curiosity that now extends to bioengineered tissues capable of regeneration and self-organization. Building on my CAD (Computer-Aided Design) foundation, I designed and microfabricated multiple STAMP iterations (Fig. 2) using nano 3D printing, enabling design flexibility. Each design features rectangular alignment grooves (25  $\mu\text{m}$  depth and width) and cross-shaped protrusions, serving as reference points to map muscle fibers across imaging sessions and modalities (e.g., muscle contraction videos and fluorescence microscopy of antibody stained tissues). Six STAMP variants (referred to as A–F) were fabricated with differing cross geometries and three height conditions (60, 90, and 120  $\mu\text{m}$ ). Cross features ranged from 30–120  $\mu\text{m}$  in width and 900–1500  $\mu\text{m}$  in length, designed to form deep gel indentations where C2C12

myoblasts are expected to develop alignment patterns identifiable by microscopy (Fig. 3-4). I then mounted these STAMPS onto custom “STAMP holders” (Fig. 7) designed to minimize slender support fracture while optimizing the extraction force for all 96 units, skills I learned in our Mechanics and Materials course. Using multi-channel pipettes to load the 96-well plate, the holders enabled mass hydrogel patterning within minutes. Next, I optimized the composition of the cell culture substrate, the hydrogel, to ensure it would retain STAMP microtopography. Hydrogels are 3D networks of hydrophilic polymers formed through crosslinking, where polymer chains are joined via hydrogen and covalent bonds. These polymers originate from monomers composed of simple sugars, amino acids, and nucleotides. In this study, hydrogels were formed using fibrinogen and thrombin, respectively a protein and an enzyme. Following the protocol in [5], fibrinogen concentration was tuned to control hydrogel stiffness, while thrombin concentration governed crosslinking kinetics. I determined the optimal fibrinogen concentration to be 12 mg/mL with an optimal ratio of 30 to 1, fibrinogen to thrombin. This resulted in a compliant hydrogel layer that maintained pattern fidelity.

## Results

As shown in Fig. 3, the optimized hydrogel retained small features (alignment grooves) and large indentations (crosses). However, after seeding C2C12 cells onto patterned hydrogels and allowing 4 days for maturation, the visibility of the cross-shaped markers varied considerably (Fig. 4). Contrary to expectations, larger crosses (A1-A3) did not yield clearer patterns; smaller crosses such as C3 and F3 produced more distinct outlines. The depth of the cross structures proved more influential than lat-

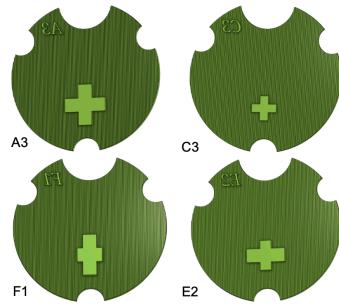


Figure 2: Selected CAD renderings of STAMPS with different cross designs.

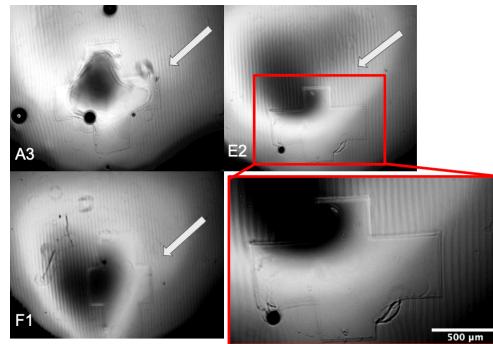


Figure 3: Corresponding hydrogel microscopy imaging for STAMPS in Fig. 2.

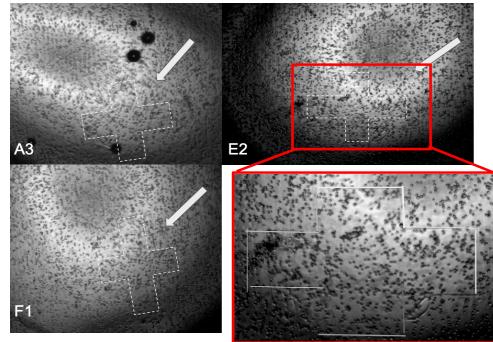


Figure 4: Corresponding hydrogel microscopy imaging with cells for STAMPS in Fig. 2.

eral dimensions. Due to hydrogel elasticity, engravings formed sloped contours instead of sharp ridges, indicating partial edge collapse. Consequently, marker visibility correlated primarily with cross height rather than width. To address this, I redesigned the STAMPS with improved topography: deeper cross markers, perpendicular alignment grooves at the surface of the crosses, and recessed cavities in place of protrusions (Fig. 5). These changes visibly



Figure 5: New STAMP designs with deeper crosses, perpendicular grooves, and cavities.

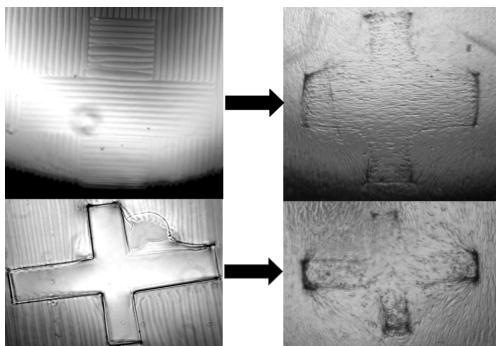


Figure 6: Hydrogel patterning from STAMPS in Fig. 5 pre and post cell seeding.



Figure 7: 12 x 8 STAMP holder

patterned hydrogels, with markings that remained apparent after one day of cell maturation (Fig. 6), and are expected to enhance post cell growth visual clarity following continued cell growth for downstream force analysis.

## Conclusion

I successfully adapted the STAMP design to pattern 96 wells with microtopographical features in a single step. Although uniform protrusions under 200  $\mu\text{m}$  became nearly invisible after cell growth, I refined my STAMP designs to improve marker contrast and fi-

delity that featured promising results with one day of cell maturation. These advancements bring this project closer to realizing a fully integrated 96-well muscular assay capable of generating high-throughput contractile force datasets—an important step toward scalable preclinical testing for neuromuscular disease therapeutics.

## Future Studies

Future work will focus on scaling the optimized STAMP system for commercial and clinical applications. This means moving away from error-prone steps, such as mounting STAMPS to holders, which is currently done manually. I am developing a modular STAMP alignment grid that uses kinematic coupling to secure all 96 STAMPS precisely. This design will enable automated handling—allowing robotic systems or technicians to mount entire arrays in a single step. Beyond manufacturing, future studies will integrate live-force imaging, automated drug screening, and computational models of muscle contraction, paving the way for predictive, high-throughput neuromuscular diagnostics.

## References

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