INTRODUCTION
Cardiovascular disease (CVD) affects more than 71 million Americans accounting annually for nearly $400 billion in US health care costs. [1] In adult tissues such as the heart, the capacity for self-regeneration is limited. Recently, stem cell therapy has emerged as a promising methodology for myocardial repair.

In 2002, Zimmermann showed that mechanical stimulation is important for engineering 3D cardiac tissue from rat neonatal cardiomyocytes.[2] Two years later, Radisic showed that electrical stimulation is also important for engineering 3D cardiac tissue.[3] In 2006, the Zimmermann group extended their work and showed that their cardiac tissue grafts improved systolic and diastolic function in infarcted rat hearts.[4] while Guo demonstrated the fabrication of a 3D cardiac tissue graft from mouse stem cell-derived cardiomyocytes.[5]

Our long-term goal is to study the effects of combining electrical and mechanical stimulation and extend these previous studies to creating an improved stem cell-based cardiac tissue graft. As a first step in this process, our goal was to create a pulsatile pressure system for applying mechanical stimulation to cells arranged in a 3D configuration, which we describe below.

MATERIALS AND METHODS
Experimental Setup
P19 mouse stem cells, Matrigel, Types I collagen, and mechanical stimulation were combined to engineer a 3D tissue graft.

The above constituents were poured into cylindrical molds with silicone tubing at the center of the molds (Figure 1, Left). The use of silicone tubing allowed the formation of 3D tissue grafts. After allowing the constituents to form from Days 0-10 in static culture, the silicone tubing was removed from the molds and the formed cylindrical tissues were moved to the center of the silicone tubing (Figure 1, Right). At this point, the silicone tubing-cylindrical tissue graft was connected inline to a pulsatile flow system (Figure 2), where it underwent cyclic strain from Days 10-18 via application of gradually increasing pressures and flows from 125 mmHg, 100 mL/min to 250 mmHg, 150 mL/min at a nearly constant rate of 1.2 Hz. Media was circulated in and around the tissue grafts (Figure 3, Left) to provide additional nourishment.

Predicted Strain
The silicone tubing-cylindrical tissue graft system was modeled in order to estimate its yield stress and estimated circumferential strain from a given pressure gradient. Material properties for silicone were used in conjunction with estimated values for the tissue graft. To determine strain, the elastic modulus of silicone and the tissue graft were calculated using the relation of shear modulus, G, Poisson’s Ratio, v, and elastic modulus E as given below:
Using a $v = 0.5$ for both materials, $E$ was calculated as the following:

$$E = 2G(1 + v)$$  \hspace{1cm} (1)

The system was modeled as composite with material properties in proportion to the sectional area of each of its components. The circumferential stress was calculated as

$$\sigma_c = \frac{P_t R_0}{T_0}$$  \hspace{1cm} (3)

Using Laplace’s equation and modeling the silicone tubing-cylindrical tissue graft system as a Hookian system the change in radius was determined by the following relation between initial inner radius, $R_0$ transmural pressure difference, $P_t$, composite elastic modulus, $E$, and initial wall thickness, $T_0$:

$$\frac{R_a}{R_0} = \frac{P_t R_0}{E T_0} \left( \frac{R_a}{R_0} \right)^v + 1$$  \hspace{1cm} (4)

Rearranging the following equation and solving the quadratic yields the following equation:

$$R = R_0 \left( \frac{P_t R_0}{E T_0} \pm \sqrt{\frac{P_t^2 R_0^2}{4 E T_0^2} + 1} \right)$$  \hspace{1cm} (5)

From this the predicted strain was calculated and we verified that the applied pressure was causing a circumferential stress significantly below the system yield stress.

Our experimental strain was determined by video image analysis (LabView 8.0) where edge-detection was used to track pixel intensity changes of the silicone tubing-cylindrical tissue graft system over several cycles.

RESULTS

Our grafts remained viable from Days 0-18 as assessed by daily microscopic inspection. In addition, over the dynamic culture period, cells could be seen proliferating and migrating away from the tissue graft along the silicone tubing (Figure 3, Right).

Figure 3. (Left) Tissue grafts in pulsatile chamber with internal and external flow. (Right) Magnified view (10X) of tissue graft around silicone tubing.

The strain resulting from application of gradually increasing pressures and flows from 125 mmHg, 100 mL/min to 250 mmHg, 150 mL/min (at 1.2 Hz) was 0.5 % and 1.6 % as shown in Figure 4, which were below the predicted strain of 4%.

Further histological data is currently being performed on both strained and unstrained tissue grafts to determine if any differences resulted from mechanical stimulation.

CONCLUSIONS

A 3D tissue graft was successfully constructed using P19 mouse stem cells, Matrigel, Types I collagen, and mechanical stimulation. Our experimental strain increased with increasing pressure and flow, however, our predicted strain was approximately 2.5 times greater than our experimental strain, which may have been due to an underestimation of our composite elastic modulus. Work is under way to improve our model.

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REFERENCES