Simulating muscular thin films using thermal contraction capabilities in finite element analysis tools

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Abstract

In this study, new techniques for approximating the contractile properties of cells in biohybrid devices using Finite Element Analysis (FEA) have been investigated. Many current techniques for modeling biohybrid devices use individual cell forces to simulate the cellular contraction. However, such techniques result in long simulation runtimes. In this study we investigated the effect of the use of thermal contraction on simulation runtime. The thermal contraction model was significantly faster than models using individual cell forces, making it beneficial for rapidly designing or optimizing devices. Three techniques, Stoney's Approximation, a Modified Stoney's Approximation, and a Thermostat Model, were explored for calibrating [thermal expansion/contraction parameters \(TECPs\)](#) needed to simulate cellular contraction using thermal contraction. The [TECP](#) values were calibrated by using published data on the deflections of muscular thin films (MTFs). Using these techniques, [TECP](#) values that suitably approximate experimental deflections can be determined by using experimental data obtained from cardiomyocyte MTFs. Furthermore, a sensitivity analysis was performed in order to investigate the contribution of individual variables, such as elastic modulus and layer thickness, to the final calibrated [TECP](#) for each calibration technique. Additionally, the [TECP](#) values are applicable to other type of biohybrid devices. Two non-MTF models were simulated based on devices reported in the existing literature.

Keywords: Biohybrid, finite element analysis, modeling, thermal contraction, muscular thin films

1. Introduction

Research into the development of biohybrid devices and actuators has become an area of increasing study. Such devices are fabricated by seeding contractile cells (skeletal muscle cells [6, 27], cardiomyocytes [10, 8, 21, 26, 5], or vascular smooth muscle cells [1, 12]) on a biocompatible substrate. The actuation of the cells then deforms the substrate, resulting in motion. Biohybrid devices have been produced that can deform micropillars [18], or locomote via

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swimming [21, 26], or crawling [5, 15, 6]. Additionally, muscular thin films, devices composed of a biocompatible cantilever seeded with cardiomyocytes, have been developed to serve as lab-on-chip setups which allow researchers to measure cellular contractile force and test the effect of chemical agents on cellular performance [23, 1]. Such biohybrid devices have great potential in the development of soft robotics, medical devices, and rapid throughput pharmaceutical testing.

As the field of biohybrid devices grows, engineering models are needed to help researchers design and simulate the effect of cellular contraction on substrate deformation. Such devices should be computationally efficient and enable new researchers to enter the field easily. Many modeling techniques for soft bodied devices employ Finite Element Analysis (FEA). In simulating biohybrid devices, current models implement cell forces via truss elements [3], cell-substrate interface traction forces [23], or use complex constitutive equations [24]. Such techniques often result in long simulation runtimes, and implementation of complex constitutive equations serves as a barrier of entry for new researchers. To overcome such barriers, we investigate the possibility of simulating cellular contraction by using the thermal strain capabilities readily available in FEA packages. Previous studies have used thermal contraction to simulate the whole myocardium [11] as well as muscular thin films [20]. However, Na et al. [20] do not discuss or compare techniques used for calibrating the required thermal contraction coefficient of related temperature changes used in their FEM simulations. In addition, they have not investigated their undisclosed calibration technique for applicability to models beyond muscular thin films or for sensitivity to material properties and device geometries. In comparison, in this study, we compare three techniques for calibrating a combined thermal property parameter: the thermal expansion/contraction parameter ($TECP = \alpha\Delta T$). In order to approximate cellular contraction using thermal contraction capabilities, a $TECP$ which will generate deformations that are equivalent to those produced experimentally by the contractile force of cells must be calibrated.

In this study, we investigate three techniques for calibrating $TECP$ values: Stoney’s Approximation [25, 19], a Modified Stoney’s Approximation (MSA) suggested by Atkinson [2, 16], and a traditional Bilayer Thermostat Model [22]. Additionally, a sensitivity analysis has been performed on each technique in order to investigate the effects of specific variables on the resulting $TECP$ calibration. Furthermore, the calibrated $TECP$ values have been applied to non-MTF devices in order to investigate the applicability of the $TECP$ values beyond the calibration set. Finally, the use of thermal contraction vs. individual cell force modeling has been explored in order to demonstrate the computational simulation time of the thermal modeling technique.

2. Methods

In the development of biohybrid actuators, many studies have implemented thin, biocompatible cantilevers with contractile cells seeded on one surface of the device. Such devices, often known as muscular thin films (MTFs), bend

in response to the cellular contraction (Figure 1). The stress in the cell layer can then be calculated based on the deflection of the cantilever. In this study, 13 MTFs have been used in order to calibrate and validate the approximation techniques [10]. In order to simulate contraction, the resulting TECP values ($\alpha\Delta T$) are always negative so that the thermal gradient results in compressive strains, representing contraction. In FEA this can be implemented as a positive and a negative ΔT , or a negative and a positive ΔT . Both combinations result in a negative TECP value and produce the same contractile effect. Additionally, two non-MTF devices [6, 5] have been used for investigating the applicability of the calibrated TECP values to biohybrid robots previously reported in the literature. The geometric and material properties for the MTFs, and both biohybrid robots are reported in Table 1.

Figure 1: A: Schema of the MTF cantilevers. A biocompatible cantilever is supported by a fixed boundary condition (B.C.) and a thin cell layer is seeded on the surface. Contraction in the cell layer causes the substrate layer of the MTF to bend upwards. B: Experimental image of 'Heart-on-a-chip' muscular thin films in their minimum contracted state (diastole). C: Experimental image of 'Heart-on-a-chip' muscular thin films in their maximum contracted state (systole). In B and C solid blue outlines indicate the original shape of the PDMS film while capped red bars indicate the projection of the thin film during contraction.

2.1. "Heart-on-a-chip" Muscular Thin Films

The multi-step manufacturing procedure of the heart-on-a-chip is fully described by Knight et al. [17]. Briefly, a sacrificial polymer, poly(N-isopropylacrylamide) (PNIPAM) (Polysciences, Inc., Warrington, PA) is spin-coated in strips onto a large glass cover-slip [10]. Polydimethylsiloxane (PDMS) (Ellsworth Adhesives, Germantown, WI) is then spin-coated on top in a uniform layer, and cured overnight in a 60°C chamber. The large glass is then separated into smaller cover-slips that will fit either a six-well or a 12-well standard plate. Fibronectin is then micro-contact printed onto the films in any desired pattern [17], specifically here 20 μm lines with 5 μm spacing.

Neonatal rat ventricular myocytes are harvested from 2-day old rat pups (Charles River Laboratories, Wilmington, MA) [7] in accordance with the Institutional Animal Care and Use Committee of UCI guidelines (Animal Experimentation Protocol permit number 2013-3093-0). The harvest procedure is laid out in detail in [7]. The cells are then seeded onto the chips at a density of 10^6 cells per 3 ml. The cardiomyocytes are cultured as described by Knight et al. [17], and then the heart-on-the-chip experiments are performed. The procedure for recording movies of the deformation of films is described thoroughly in [10, 17]. The heart-chip is prepared for the contractility experiments with rows of rectangles being cut into the PDMS, which gives water access to the sacrificial polymer. As the sacrificial polymer dissolves away, the films lift up, except at one edge. Thus the films are hinged and will bend away from the glass surface during cardiomyocyte contraction (Figure 1.B and C). The films are paced at 2 Hz (MyoPacer Field Stimulator - IonOptix, Milton, MA), and their deformation is imaged from above using a Basler A601f/A602f camera (Basler, Inc., Exton, PA) mounted on a stereo-scope (Olympus SZX-ILLB2 - Olympus America, Center Valley, PA). The films are tracked using custom ImageJ and MatLab codes [10].

2.2. Leave-k-Out Cross Validation

Based on MTF experimental results, three techniques have been investigated for calibrating **TECP** values for use in finite element approximation of cellular contraction. Due to passive cell stresses causing some deflection of the MTF, **TECP** values were calibrated for both the minimum and maximum radius of curvature. For each calibration technique, a Leave-k-out cross-validation has been employed[14]. The thirteen experimental samples were divided into a training set comprised of 80% of the original samples (10 samples), and a validation set made up of the other 20% (3 samples) for a Leave-3-out analysis. This resulted in 286 calibration/validation sets. Each sample in the calibration set was used to calibrate the **TECP** value independently and then the mean **TECP** value for that set was determined. This was repeated on all combinations possible for dividing the original sample. The mean values was subsequently used for model validation using the remaining 3 samples in each set (Figure 2).

2.3. Model Calibration

2.3.1. Stoney's Approximation

The first calibration technique relies on the application of Stoney's equation [25, 19]. Stoney's equation relates the radius of curvature of a cantilever comprised of substrate and thin film layers to the stress in the thin film:

$$\sigma_c = \frac{1}{6Rt_c} \left(\frac{E_s t_s^3}{(1 - \nu)(t_s + t_c)} + \frac{E_c t_c^3}{(1 - \nu)(t_s + t_c)} \right) \quad (1)$$

where R is the experimentally measured radius of curvature of the beam, t_c and t_s are the thicknesses of the cell and substrate layers respectively, and E_c and E_s are the moduli of the cell layers, respectively. The Poissons ratio,

Figure 2: A diagram of the model calibration/validation process. Experimental results are analyzed in order to determine the radius of curvature of the cantilever. This value is then used by the three calibration techniques to determine the **TECP**. In this study, only the MSA and Thermostat model have been used for validation, as no significant difference in calibrated **TECP** was found between the Stoney's Approximation and MSA.

ν was assumed to be the same for both the substrate and cell layers based on values previously reported in the literature [20, 11, 23]. The **TECP** was then determined from the cell layer stress using the linear thermal stress-strain relationship:

$$\alpha \Delta T = \frac{\sigma_c}{E_c} \quad (2)$$

Table 1: Material properties of the experimental muscular thin films and several biohybrid devices previously reported in the literature.

Model	E_s (kPa)	E_c (kPa)	ν
MTF[10]	1.5×10^3	30	unknown
Chan[5]	17.8-503	10	0.499
Cvetkovic[6]	214-489	unknown	unknown
Park[23]	750	188	0.49
Feinberg[8]	1500	30	unknown

Table 2: Geometric properties of the experimental muscular thin films and several biohybrid devices previously reported in the literature.

Model	t_s (μ)	t_c (μm)	w (mm)	L (mm)
MTF[10]	1.5×10^3	30	varies	2.6-3.1
Chan[5]	450	10	2	4
Cvetkovic[6]	N/A	N/A	3	N/A
Park[23]	20	4.5	0.05-0.15	0.25-0.75
Feinberg[8]	30	5	10-20	5-10

2.3.2. Modified Stoney's Approximation

Stoney's original approximation is limited in that the accuracy decreases as the thickness ratio between the thin film and the substrate increases. However, a modification to Stoney's original approximation has been developed which allows analysis of cantilevers with thickness ratios up to 30% [16]. This results in the following expression for the stress in the cell layer:

$$\sigma_c = (1 + \delta^3 \gamma) \frac{1}{6Rt_c} \left(\frac{E_s t_s^3}{(1 - \nu)(t_s + t_c)} + \frac{E_c t_c^3}{(1 - \nu)(t_s + t_c)} \right) \quad (3)$$

where the additional correction factor contains $\delta = \frac{t_c}{t_s}$ and $\gamma = \frac{E'_c}{E'_s}$ and $E' = \frac{E}{1 - \nu}$.

Again, the **TECP** was determined by using the linear thermal stress-strain relationship (Eq. 2).

2.3.3. Thermostat Model

The final calibration techniques considers the muscular thin film in the context which it will be approximated in the finite element model. That is, the calibration technique assumes that the MTF is a bilayer cantilever with each layer having a unique thermal expansion coefficient, as in a thermostat [22]. In this case, the substrate is assumed to have a null thermal expansion coefficient $\alpha = 0$. As a result, the **TECP** of the cell layer can be calculated directly as:

$$\alpha \Delta T = -\frac{1}{6R} \frac{(E_s t_s^2 - E_c t_c^2 + 4E_s E_c t_s t_c (t_s + t_c^2))}{E_c t_c (E_s t_s t_c + E_s h_s^2)} \quad (4)$$

2.4. Sensitivity Analysis

In order to investigate the effect of individual geometric and material properties on each calibration technique a one-at-a-time (OAT) sensitivity analysis [13] has been performed on each calibration technique by varying E_c , E_s , t_c , t_s . Initially, each variable was individually varied to the minimum and maximum values found in existing literature (see Table 1 and 2) and the resulting change in the calibrated **TECP** was determined. Additionally, each technique was analyzed by individually varying E_c , E_s , t_c , t_s , and the radius of curvature across a range of appropriate values around the base value from the MTF experimental results.

2.5. Model Validation

2.5.1. Muscular Thin Films

For each combination, the training set was used for calibration using the three calibration techniques. After calibrating the **TECP** values using each of the three techniques described above, it was found that the usage of Stoney’s Approximation versus the MSA did not result in statistically significant differences in the calibrated **TECP** values (See Section 3.1). Therefore, only the MSA and the Thermostat model were used for model validation. The mean **TECP** value for each was used to simulate the geometries in the validation set.

2.5.2. Biohybrid Robots

The calibration techniques were also tested for their ability to generalize to additional types of biohybrid devices. Due to the sensitivity of the calibration techniques to substrate modulus (see Section 4.1), the calibrated **TECP** values are most likely to generalize to devices with similar moduli. Two biohybrid robots previously reported in the literature were used for additional validation [6, 5]. For both devices, 100 mean **TECP** values were randomly selected from the maximum contracted state validation sets and used to simulate the biobots. The first is based on the walking biobot reported by Cvetkovic et al. in 2014 [6], which is composed of a printed PEGDA spine between two pillars. A tissue construct is then cultured between the pillars such that contraction of the tissue bends the spine (Figure 3.A). The substrate modulus varied from 214.6 kPa to 489.3 kPa. This had been implemented in ANSYS as 2-D symmetric model, and the simulated force in the cell layer was compared to the reported values.

The second model is based on the biohybrid robot reported by Chan et al. in 2012 (Figure 3.B) [4]. This device is an extension of the MTF geometry, where deflection of a cantilever arm pulls the biobot along in a crawling gait. The cantilever arm was simulated in ANSYS using 2D plane stress elements, and the simulated deflection was compared to the reported experimental values.

2.6. Simulation Runtime

The effect of using thermal contraction, rather than individual forces for cells, on simulation time was investigated by simulating a 10 mm long MTF either using the thermal contraction technique or by simulation of cellular contraction by applying individual cell forces at the interface between the cell and substrate layer at every 100 μm . Such a long MTF was simulated in order to highlight the difference in simulation runtimes for larger models. A batch simulation technique was used wherein the simulation was run 200 times from MATLAB and the elapsed time for each simulation was recorded. Prior to the statistical analysis, outliers that were 1.5 times the interquartile range above third quartile were removed.

2.7. Transient Simulation

Using this thermal contraction technique, it is also possible to perform transient analyses of the MTFs. For transient simulations, the ANSYS simulation

Figure 3: A: Schema of the biohybrid robot from Cvetkovic et al 2014 [6] (Left), which is modeled as a symmetric shape with the **TECP** value applied to the tissue construct (Middle). The device is then simulated in ANSYS (Right). B: Schematic of the biohybrid robot from Chan et al. 2012 [4] (Left), which is simplified to a MTF model of the front arm (Middle). The device is then simulated in ANSYS (Right). In both cases a fixed displacement boundary condition was implemented along the left edge as indicated in the middle figure. This boundary condition allowed rotation of nodes but no displacement.

was performed with $\alpha = 1$ and the time varying **TECP** input as a table of temperatures. As with the static analysis, **TECP** values were calibrated based on experimental radii of curvature. However, **TECP** values were calibrated for each time point of each calibration set rather than just the minimum and maximum. Because the Thermostat model was found to best capture the deflection of the MTFs (See Section 3.3.1), transient simulations were only performed using **TECP** values calibrated using the Thermostat technique. For each calibration set, the **TECP** mean value at each time point was determined to populate the temperature table. One validation sample from each set was selected from each set for simulation. The deflections of the simulated results containing the maximum and minimum deflection values were compared to the experimental deflections containing the maximum and minimum deflection values.

2.8. Statistical Analysis

The **TECP** values calibrated using each technique were compared using one-way ANOVA for both the minimum and maximum radius of curvature calibrations, both of which had normal distributions. Individual calibration techniques were then compared using a two-sample t-test. Since multiple comparisons were being made, a Bonferroni correction was applied, resulting significance being determined at $p < 0.0167$.

For analyzing the effect of modeling technique on simulation runtime (normal distribution) a two-sample t-test was used with significance being determined

Figure 4: A. The MTF model simulated in order to determine the average simulation time for the thermal contraction approximation. The cell layer is given a **TECP** value while the substrate layer has no thermal expansion or contraction. B. The MTF model used to determine the average simulation time of the individual cell force model. Each cell applies forces to the connection between the cell and substrate layer every 100 μm .

at $p < 0.05$.

3. Results

3.1. Model Calibration

By utilizing a leave-3-out cross validation technique with 13 original samples, the calibration technique resulted in 286 unique combinations. For each set, 10 **TECP** values were calibrated, resulting in a total of 2860 **TECP** values for each calibration technique. The **TECP** distributions were compared for each technique using ANOVA followed by two-sample t-tests. Statistical analysis revealed that there was no significant difference in the calibrated **TECP** values between the Stoney's Approximation and MSA, but that the Thermostat Model resulted in significantly different values from either ($p < 0.0167$).

Figure 5: A box and whisker plot of the calibrated **TECP** for the minimum contracted state (Left) and maximum contracted state (Right) for each of the three calibration techniques. The boxes indicate the 1st and 3rd Quartile while the bars represent the minimum and maximum values.

The use of Stoney's Approximation, the MSA and the Thermostat Model to calibrate a **TECP** value from the minimum contracted state resulted in **TECPs** of -0.220 ± 0.004 , -0.219 ± 0.004 , and -0.147 ± 0.003 , respectively, whereas

Figure 6: E_c , E_s , t_c , t_s were varied to the minimum (red dotted outline) and maximum (black outline) values found in the literature. The resulting % change in the calibrated **TECP** value was determined. In the range of values reported in the literature for MTFs. The cell modulus resulted in the largest change in calibrated **TECP**

for the maximum contracted states, the **TECP** values for the use of Stoney's Approximation, the MSA and the Thermostat Model resulted in **TECPs** of -0.387 ± 0.017 , -0.387 ± 0.017 , and -0.259 ± 0.012 , respectively.

3.2. Sensitivity Analysis

In addition to comparing the simulation results to the experimental deflections, a sensitivity analysis was performed on all three techniques in order to investigate the effect of individual variables on the calibrated **TECP** value. The values were varied to the maximum and minimum value reported in the literature (Figure 6).

Additionally, individual variables were varied over a wide range in order to investigate the effect on calibrated **TECP** (Figure 7). In lower values, cell thickness exhibits the largest effect, whereas with increasing substrate thickness, the substrate thickness results in larger effects.

3.3. Model Validation

3.3.1. Muscular Thin Films

The mean **TECP** for each calibration set was then determined and the geometric conditions for each of the validation set samples were simulated using their respective mean **TECP** for both the MSA and Thermostat Model. The resulting simulated deflection was then compared to the experimental results (Figure 8).

Figure 7: The individual sensitivities of **TECP** calibration as individual variables are varied. The Y axis gives the calibrated **TECP** as the variable listed on the X-axis is varied, while all other values are held constant.

Figure 8: A box and whisker plot of the experimental and simulated deflections using the minimum calibrated **TECP** (Left) and maximum calibrated **TECP** (Right). The boxes indicate the 1st and 3rd Quartile while the bars represent the minimum and maximum values. Although the Thermostat Model better predicts the experimental results, the predicted distributions are still significantly different than the experimental results in all cases. The best approximation came from the Thermostat Model's prediction of diastole ($p = 0.013$)

3.3.2. Biohybrid Robots

In order to investigate the applicability of the calibrated values to additional biohybrid devices, two biohybrid robots previously reported in the literature were simulated and the simulated results were compared to the experimental values. The first model, based on Cvetkovic et al.'s two legged biohybrid walker [6], was analyzed for the active stress in the tissue construct. The Thermostat method was found to better predict the active tension in the tissue construct

(37% error) than the MSA method (102% error) as shown in Figure 9.A.

The second model is based on the biohybrid crawling robot reported by Chan et al. in 2012 [4]. The arm of this device was simulated and the resulting deflection was compared to the reported deflection over the course of the devices lifetime (specifically at approximately 55, 75, and 95 hours post seeding). Comparison of the predictions of the MSA and Thermostat methods with experimental deflection indicated that the Thermostat method was in greater agreement with experimental results than the MSA method (Figure 9.B). For the 75 and 95 hour time points the Thermostat Model had a relative error of -13% and 4.2% respectively, whereas the MSA method had errors of 30% and 56%, respectively.

Figure 9: A comparison of experimental and simulated results for biohybrid devices based on work by Cvetkovic et al. [6] (A), and Chan et al. [5] (B). For the device presented by Cvetkovic et al., the experimental results were reported as a single value of active tension in the tissue construct. For the device presented by Chan et al., the experimental results were reported as the amplitude of deflection at 55, 75, and 95 hours post initial cell seeding. The simulated results are presented as a box and whisker plot. The boxes indicate the 1st and 3rd Quartile while the bars represent the minimum and maximum values. In both cases, the MSA method overestimates the experimental results. However, for the device presented in B, while the MSA method still overestimates the experimental deflection, the Thermostat Model successfully predicts values in the range of the experimental results.

3.4. Simulation Runtime

In order to compare the use of a thermal contraction approximation to the use of individual cell forces, large simulations of each type were run 200 times and the average time for each technique was determined (after removal of outliers). The use of individual cell forces resulted in an average simulation time of 339.2 ± 1.2 seconds, whereas the use of the thermal contraction approximation resulted in an average simulation time of 72.9 ± 0.5 seconds. As a consequence, the thermal contraction approximation was significantly faster than the use of individual forces ($p = < 0.005$).

3.5. Transient Simulation

The transient simulation is able to capture the qualitative behavior of the MTFs. Additionally, the experimental results are bounded by the simulated results calibrated for the strongest and weakest films (Figure 10).

Figure 10: A comparison of transient simulation results to the experimental results for the strongest and weakest films. The strongest and weakest experimental films are bounded by the corresponding simulation results. The strongest Thermostat Model results are indicated by the dashed blue line at the top of the plot, whereas the weakest Thermostat Model results are indicated by the dashed orange line at the bottom of the plot. The strongest and weakest experimental results are indicated by solid lines.

4. Discussion

4.1. Sensitivity Analysis

All three methods are most sensitive to the cell modulus and substrate thickness. This is important because many studies rely on previously reported values for their anticipated cell layer thickness and cell layer modulus, rather than

making measurements of the actual system. While these measurements can be difficult, they are important to ensure that the stress in the cell layer is being approximated correctly. All three techniques followed similar trends. The techniques showed increased sensitivity to cell layer modulus as the modulus decreased, whereas the sensitivity to substrate modulus was linear throughout the range of values explored. Additionally, the techniques were more sensitive to cell layer thickness at lower values, but were more sensitive to substrate thickness as the thickness increased. Finally, the techniques were more sensitive to the radius of curvature, and the thickness ratio at lower values of these parameters.

4.2. Model Validation

The use of the Thermostat calibration model significantly reduced the error in the predicted deflection. The result is unsurprising as Stoney’s Approximation relies on several key assumptions which are violated by application to MTFs [25, 9]:

1. The substrate and thin film have uniform thickness
2. The equation was developed for circular disks
3. The substrate and thin film are homogeneous, isotropic, and linearly elastic

It is unlikely that the cell layer has a completely uniform thickness, and it is certainly not isotropic in the case of patterned MTFs. Therefore, assumptions 1 and 3 would likely impact the performance of both the MSA and the Thermostat model as calibrating techniques. However, Stoney’s Approximation was initially developed for circular disks. In contrast, the Thermostat model and the experimental setup are both configured for cantilevers. It is therefore possible that the improvement of the Thermostat model over the MSA is due to the boundary conditions better approximating those of the experimental setup.

4.2.1. Biohybrid Robots

Both the MSA and the Thermostat model predict active tensions higher than the reported experimental value. However, the Thermostat model serves as a better approximation, greatly reducing the error between the prediction and the experimental value.

Unfortunately, the tissue construct modulus was not reported by Cvetkovic et al. However, due to the fact that the tissue construct contained additional components (collagen or fibrin), it is likely that the modulus was higher than that of cells alone. Based on the sensitivity analysis (See Section 4.1), a higher cell modulus would have resulted in a lower [TECP](#) value. Additionally, this device used skeletal myotubes, rather than cardiomyocytes, further complicating the applicability of the [TECP](#) values calibrated from cardiomyocyte powered MTFs.

The second model is based on the biohybrid crawling robot reported by Chan et al. in 2012 [4]. The MSA [TECP](#) values predicted the deflection to be higher than the reported experimental values, but the Thermostat model successfully

predicted deflections within the ranges of both the 75 and 95 hours experimental values.

4.3. Simulation Runtime

The use of thermal contraction rather than individual cell forces resulted in a significantly lower simulation runtime for otherwise identical MTFs. This makes the use of the thermal contraction technique more effective for rapidly designing prototype devices, as devices can be quickly simulated for geometry and material property optimization.

4.4. Transient Simulation

Using these techniques, it is possible to run rapid, transient simulations. This will allow researchers to prototype moving biohybrid devices quickly and effectively using tools with which they are already familiar. By using the Thermostat calibration technique, it is possible to obtain simulated results which bound the experimental results, thereby providing a possible range of behaviors or performance of the device. Additionally, since many FEM packages integrate with CAD software, these techniques make it possible to rapidly prototype device designs and test their dynamics.

4.5. Limitations

There are several limitations of the present study which should be addressed. First, due to the lack of nonlinear material properties reported in the literature, the present study treats both the cell layer and substrate layer as linear elastic materials. However, both biological materials and biocompatible polymers exhibit nonlinear material behaviors. While not included in the present study, it is straightforward to implement nonlinear material models in finite element analysis. This would further improve the ability of the model to predict experimental deflections of muscular thin films or biohybrid devices. Second, the present models consider only uniaxial contraction of the cells. This is due to deflections of the cantilevers or biohybrid devices from the literature only being reported along the contraction direction of the cells. However, cellular contraction exhibits biaxial behavior. While not included in the present study, such contraction could be achieved using the presented thermal contraction technique by including a coefficient of thermal expansion for the cell layer perpendicular to the contraction direction. This TECP would need to be calibrated as the contraction direction TECP was, or related to the axial TECP via constitutive equations.

5. Conclusions

This study has presented a technique for efficiently approximating the deflection of biohybrid devices and MTFs using the thermal contraction capabilities

of finite element analysis packages. Three techniques - Stoney's Approximation, the MSA and the Thermostat Model - were explored and the approximation technique based on a classic bilayer thermostat model was found to better predict MTF and biohybrid device behavior in comparison to approximations based on Stoney's approximation. Based on the results presented here, a range of [TECP](#) values of -0.147 to -0.259 can be used to approximate cellular contraction. Such techniques are significantly faster than using individual cell forces, and are applicable to devices beyond the initial calibration set. These approximation techniques reduce the barriers of entry for new researchers to the field of biohybrid robotics, allowing them to more rapidly design and analyze biohybrid devices.

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7. Appendix

7.1. Thermostat Model Simplification

The Thermostat Model calibration technique discussed above is based on treating the MTF as a bilayer cantilever wherein each layer has an individual elastic modulus and thermal expansion coefficient. For such a system the expected curvature can be calculated as:

$$\frac{1}{\rho} = -\frac{6}{D}[(\alpha_1 E_1 T_1 t_1^2 w_1 - \alpha_2 E_2 T_2 t_2^2 w_2)(E_2 t_2 w_2 + E_1 t_1 w_1) + (\alpha_1 E_1 T_1 t_1 w_1 + \alpha_2 E_2 T_2 t_2 w_2)(E_2 t_2^2 w_2 - E_1 t_1^2 w_1)] \quad (5)$$

where ρ is the radius of curvature, α_i is the thermal expansion coefficient of the respective layer (1 - cell layer, 2 - substrate layer), E_i is the elastic modulus of the layer, t_i is the thickness of the layer, w_i is the width of the layer, and D is calculated as:

$$D = (E_2 t_2^2 w_2 - E_1 t_1^2 w_1)^2 + 4E_1 E_2 t_1 t_2 (t_1 + t_2)^2 w_1 w_1 \quad (6)$$

In this MTF case, $w_1 = w_2 = w$. Additionally, we can set the thermal expansion coefficient of the substrate layer $\alpha_2 = 0$, because it is not contracting. As a result 5 and 6 simplify to:

$$\frac{1}{\rho} = -\frac{6}{D}\alpha_1 T_1 [E_1 t_1^2 w^2 (E_2 t_2 + E_1 t_1) + E_1 t_1 w^2 (E_2 t_2^2 - E_1 t_1^2)] \quad (7)$$

and

$$D = (E_2 t_2^2 - E_1 t_1^2)^2 w^2 + 4E_1 E_2 t_1 t_2 (t_1 + t_2)^2 w^2 \quad (8)$$

Solving 7 for $\alpha_1 T_1$ and substituting in D from 8 yields:

$$\alpha_1 T_1 = -\frac{1}{\rho} \frac{(E_2 t_2^2 - E_1 t_1^2)^2 w^2 + 4E_1 E_2 t_1 t_2 (t_1 + t_2)^2 w^2}{6E_1 t_1^2 w^2 (E_2 t_2 + E_1 t_1) + E_1 t_1 w^2 (E_2 t_2^2 - E_1 t_1^2)} \quad (9)$$

By assuming a base temperature $T = 0$, $E_1 = E_c$, and $E_2 = E_s$ this reduces to:

$$\alpha \Delta T = -\frac{1}{6R} \frac{(E_s t_s^2 - E_c t_c^2 + 4E_s E_c t_s t_c (t_s + t_c))}{E_c t_c (E_s t_s t_c + E_s h_s^2)} \quad (10)$$

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Graphical abstract