Virtual Particle Tracking Based In Silico Model of Engineered Heart Tissue Contractility

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Abstract

Engineered heart tissues (EHTs) provide an in vitro platform for studying cardiac physiology and disease in 3D. Accurate quantification of contraction and force generation is essential for evaluating EHT function. However, traditional methods provide only total deformation based on EHT anchor pillar deflection, with limited information about the spatial distribution of tissue deformation and contractile activity. To address this, we present a framework for EHT characterization that combines video-based motion analysis and mechanical modeling. We implemented an optical flow estimation pipeline for bright-field microscopy videos to capture motion fields during contraction. A grid of virtual particles was superimposed on the video and propagated according to the flow field, enabling the tracking of displacements through the contraction cycle. We constructed a Hill-type mechanical model, with active and passive mechanical elements representing a 2D grid corresponding to the virtual particle network, and parametrized it based on in vitro results. The model was designed to reproduce both experimentally observed motion and total contractile force generated by the tissue. This approach provides insight into the local and global EHT contraction mechanics beyond conventional force measurements, and enables a more comprehensive characterization of tissue deformation and contractility.

1. Introduction

Cardiovascular diseases remain the leading cause of death worldwide [1], thus the development of improved treatments and methods of diagnosis continue to be sought after. Engineered heart tissues (EHTs) are a novel platform for cardiac *in vitro* research as they reproduce many structural and functional characteristics of native cardiac tissue, making them a more advanced platform for studying cardiac function than traditional two-dimensional cardiac models. Thus, they are increasingly used in applications such as disease modeling, drug screening, and studies of cardiac physiology [2].

EHTs are three-dimensional cardiac tissue structures formed by culturing human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), together with supporting cell types, within a biomaterial matrix. The mixture is cast in a mold between two bending pillars. The tissue self-organizes around and between these pillars forming a dense, aligned muscle strip. The pillars provide mechanical resistance, promoting tissue maturation. Contracting tissue bends the pillars and from their subsequent deflection the total force produced by the tissue can be estimated.

While pillar deflection is used to quantify the sum of contractile forces generated by the EHTs, it provides only a limited view of the complex spatiotemporal mechanics underlying tissue contraction [3]. In particular, measurements based solely on pillar displacement do not capture regional variations in motion, deformation patterns, the distribution of mechanical activity throughout the tissue, or the actual force generated by the tissue. To address this limitation, we developed a framework that combines TV-L1 optical flow estimation [4] with a grid of virtual particles (VPs), to quantify tissue motion directly from microscopy videos of contracting EHTs. Optical flow estimation has previously been used for quantifying motion from video data, and has previously been applied to the analysis of contracting EHTs [5], by estimating the motion of pixels between consecutive frames resulting in two-dimensional displacement fields. This approach enables the assessment of local displacement dynamics across different regions of the tissue, providing a more detailed characterization of contractile behavior than conventional pillar based force measurements.

To investigate the mechanical processes governing the observed motion, and approach the estimation of active force generation, we developed a mechanical model of the

EHT corresponding to the two-dimensional VP grid. The model is designed to reproduce both the spatial motion patterns and the total contractile force measured experimentally by our video based analysis.

Similar approaches have previously been used to quantify deformation patterns [3] and to estimate regional active tension in the tissue [6]. The framework described in this paper aims at simulating EHT deformations by incorporating spatial variations in active and passive mechanical properties. By comparing simulated and measured tissue dynamics, the combined framework provides a means to evaluate how local mechanical interactions contribute to the overall contractile response of the EHT.

2. Materials and Methods

2.1. Cell culture data

The EHTs were cultured from hiPSC-CMs according to the protocol developed by Ribeiro et al. [7]. Contraction cycles were recorded using brightfield microscopy with an inverted microscope (Nikon Ti2) equipped with a Prime BSI sCMOS camera and a 2× objective at 100 fps and a 3.33 µm/pixel ratio.

2.2. Optical flow and virtual particles

In this study, tissue and pillar motion was quantified from brightfield microscopy recordings using the TV-L1 optical flow algorithm to generate velocity vector fields. TV-L1 combines total variation regularization with an L1 data fidelity term, providing dense motion estimates while maintaining robustness to image noise, illumination variations, and local motion discontinuities [4]. These characteristics make it suitable for the analysis of contractile motion in brightfield microscopy videos of EHTs.

Optical flow algorithm parameters, including the regularization parameter (λ), which controls the tradeoff between data fidelity and smoothness of the flow field, and the number of pyramid scales, which determines the range of detectable motion [4], were optimized empirically. Parameter values were selected to maximize the stability of the estimated flow fields while preserving enough detail of the motion patterns inside the tissue. The selected value of (λ) was 0.1 and the number of scales was 5.

To track the motion in different regions of the EHT, a two-dimensional grid of VPs was placed semi-automatically on top of the tissue video image. The positions of these particles were updated according to the optical flow vector field, enabling the estimation of local displacement, velocity, and the VP trajectories throughout the contraction cycle. In addition, individual VPs were placed manually on easily identifiable and trackable image features on the pillar boundaries to track their deflection and estimate entire tissue force generation. The grid of VPs can be seen in Figure 1.

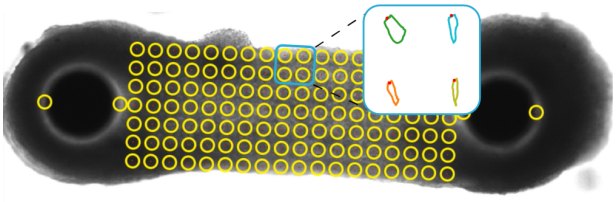

Figure 1: Virtual particle placements in brightfield video microscopy recording of an EHT and a closeup of magnified particle motion trajectories.

2.3. Mechanical model

A mechanical model was developed to characterize the relationship between local deformation, passive and active forces, and global force generation. The model was constructed here in 2D to correspond to the two-dimensional VP grid obtained from optical flow analysis, enabling direct comparison between simulated and experimentally measured tissue contraction, leaving room for future 3D imaging.

In the mechanical model, the VPs are represented as nodes connected by Hill-type mechanical elements in the horizontal, vertical, and diagonal directions, forming an interconnected two-dimensional network. Each horizontal element consists of an active contracile component in parallel with a linear spring and a dashpot (Figure 2 B), whereas vertical and diagonal components lack the active component (Figure 2 C). This allows the model to capture both active cardiomyocyte contraction and passive viscoelastic responses of the tissue. A linear spring was chosen as it reduces model complexity and parameter count, while maintaining the model's ability to capture the dominant elastic response of the tissue. The network is connected on both sides to linear springs which represent the pillars and serve as boundary conditions. Figure 2 illustrates the model architecture and its connection to VPs.

To simulate tissue deformation, the nodal motion was calculated from the sum of all forces acting on each node. To reduce model complexity, viscous effects were represented on each node using a global drag term (γ) rather than individual dashpots between nodes. Inertia effects were neglected and the velocity of each node was assumed to be proportional to the net force acting on each node. The nodal velocity was thus calculated as

$$\mathbf{v_i} = \frac{\mathbf{F_i}}{\gamma} \tag{1}$$

where $\mathbf{F_i}$ is the total force acting on the node and γ is the drag coefficient. Node positions were then updated

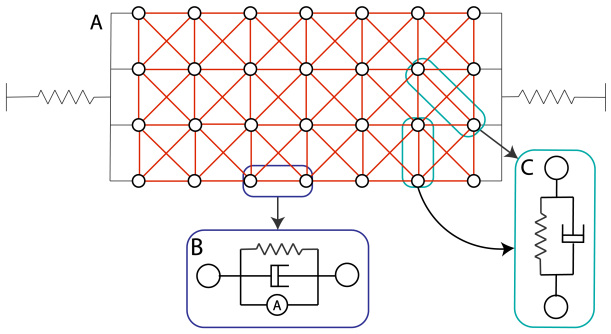

Figure 2: The mechanical EHT model. A) The nodes of the connected mechanical elements representing the virtual particle grid, B) the equivalent Hill-type mechanical models for horizontal nodal connection, and C) the models for the vertical and diagonal nodal connections.

through time integration according to

$$\mathbf{x_i}(\mathbf{t} + \mathbf{\Delta t}) = \mathbf{x_i}(\mathbf{t}) + \mathbf{v_i}\mathbf{\Delta t} \tag{2}$$

where $\mathbf{x_i}$ is the node position and Δt is the simulation time step. The tissue was assumed to be homogeneous and the spring constant was calculated from the estimated Young's modulus from in-vitro experiments. In our demonstration here, the active tension generated by the contractile elements was obtained from the model developed by Forouzandehmehr et al. [8] which was then scaled to the cross-sectional area of the elements. To simplify the model, active tension was assigned only to the horizontal elements depicting the main contraction direction. The drag coefficient was selected to provide stable numerical behavior while maintaining physiologically plausible tissue motion.

3. Results

3.1. Virtual particle estimation

Optical flow estimation combined with VP tracking enabled the characterization of tissue motion throughout the contraction cycle. The trajectories of the VPs revealed spatial variability in tissue motion across different regions of the EHT. Figure 3 presents the particle displacements in both the direction parallel to the pillar movement and in the perpendicular direction. As expected, the largest displacements were observed along the pillar motion direction, corresponding to the direction of contraction. However, non-negligible and somewhat non-symmetric perpendicular motion was detected demonstrating that axial force measurement does not fully recapitulate the contractile behavior. Figure 3 exhibited asymmetric perpendicular motion, with displacements occurring predominantly toward one direction rather than being evenly distributed around the centerline, pointing towards different regions of the tissue contributing unequally to the overall contraction and volume preservation.

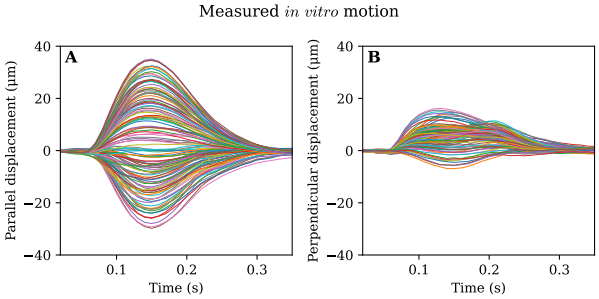

Figure 3: Traced particle motion for each particle (A) in the direction of pillar motion and (B) perpendicular to pillar motion.

3.2. Simulated motion

Simulations with the mechanical model were performed to investigate its ability to reproduce experimentally observed motion patterns. When a spatially uniform active tension signal was applied, the resulting motion was symmetric around the centerline in both parallel and perpendicular directions, as illustrated in Figure 4 A and B.

To induce asymmetric deformation, a nonuniform active tension signal was applied, with amplitudes of 80% and 90% of the original signal strength in the top row and the row immediately below it, respectively, while the remaining rows were left unchanged. This change in parameters resulted in asymmetric motion in the perpendicular direction similar to the experimental data. These results are shown in Figure 4 C and D.

The simulations were highly sensitive to the assigned active tension distribution, and further refinement of the model is required to achieve a more accurate prediction of tissue motion.

4. Discussion and conclusion

We combined optical flow-based quantification leveraging virtual particles (VPs) with a mechanistic model to better understand EHT motion dynamics. Traditionally, the EHT platform has been used for determining the axial force required for bending the platform pillars, however, that approach does not fully take advantage of the platform, nor does it enable extracting the actual active force exerted by the cells. Our study and results represent a step towards more holistic view of the EHT platform.

Tracking the EHT contraction via VPs enabled the detailed quantification of non-symmetric transverse motion.

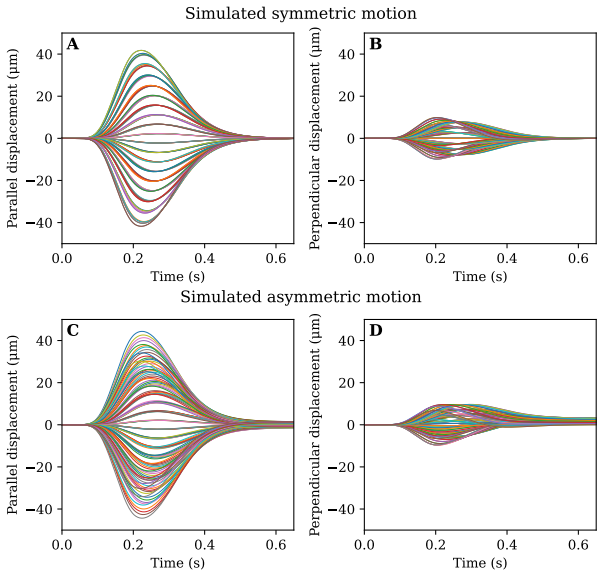

Figure 4: The simulated overall distribution of nodal (A) parallel and (B) perpendicular motion for the symmetric model parameters, and (C) parallel and (D) perpendicular motion with reduced active tension parameters in the upper region of the EHT.

This asymmetry could originate from several EHT characteristics such as distributions of different cells, cell density, myofibrillar orientation, maturity, calcium dynamics and energetics. Together, these contribute towards active components from contraction mechanics and passive components due to tissue stiffnesses.

While in our results the bending was close to the center of the tissue, assymmetry of the tissue due to defects such as cardiomyocyte distribution or fibrosis might be seen as asymmetry nearer to the poles. These results highlight the heterogeneous nature of EHT contraction and demonstrate that local deformation patterns cannot be fully characterized by pillar deflection measurements alone. While pillar motion provides a global measure of contractile force generation, VP tracking reveals quantifiable regional differences in tissue motion and deformation.

The developed biomechanical model was capable of reproducing the transverse motion. While the model reproduced the general motion patterns observed experimentally, it remains a simplified representation of EHT mechanics. Prior information about transverse passive forces in EHTs is scarce and to characterize this better, more data on their structure is needed, preferably in 3D throughout the EHT.

A limitation of the proposed method remains with using 2D video based methods in 3D culture. Biomechanical model-wise, the limitation is trivial as it can be applied to a 3D mesh similarly as in 2D, but the motion quantification in 3D across the z-axis for VP placement requires more advanced imaging methods.

The VP-based assay and the model both provide new information on the EHT motion and force generation. Together, they have potential in pharmaceutical assays and cardiac disease models, such as hypertrophic cardiomyopathy, that benefit from greater understanding of the structure-function relation in contractile mechanics.

Acknowledgments

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