Supplemental Movie 1: Visualization of Propagating Activation. Static activation thresholds are calculated for each pixel, shown as the surface in red. For each pixel, when the fluorescence intensity exceeds the activation threshold, it is assigned a different color, indicating its activation time. Thus, each individual color is an isochrone. The *z*-value for each activated pixel indicates fluorescence (a.u.). *x*- and *y*-axes indicate pixel number, micron to pixel ratio is 26 μm/px.

Supplemental Table 1: Cell Density

All cell density data were normally distributed as determined by Lilliefors' test. Significant values are bold and starred. Values with p>0.1 are written as NS (Not Significant).

Comparison	**Statistical Test**	***p*-value**
Subset: Isotropic Tissues	One-way ANOVA	<0.09
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	<0.10
Soft PDMS vs Gelatin	Fisher's LSD	**<0.04***
Subset: Aligned Tissues	One-way ANOVA	NS
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	NS
Soft PDMS vs Gelatin	Fisher's LSD	NS
Subset: Stiff PDMS, Isotropic vs Aligned	Student's t-test	NS
Subset: Soft PDMS, Isotropic vs Aligned	Student's t-test	**<0.02***
Subset: Gelatin, Isotropic vs Aligned	Student's t-test	NS

Supplemental Table 2: Cell Alignment

All cell alignment data were normally distributed as determined by Lilliefors' test. Significant values are bold and starred. Values with p>0.1 are written as NS (Not Significant).

Comparison	**Statistical Test**	***p*-value**
Subset: Isotropic Tissues	One-way ANOVA	NS
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	NS
Soft PDMS vs Gelatin	Fisher's LSD	NS
Subset: Aligned Tissues	One-way ANOVA	<0.0003
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	**<0.0004***
Soft PDMS vs Gelatin	Fisher's LSD	**<0.0003***
Subset: Stiff PDMS, Isotropic vs Aligned	Student's t-test	**<0.0001***
Subset: Soft PDMS, Isotropic vs Aligned	Student's t-test	**<0.0001***
Subset: Gelatin, Isotropic vs Aligned	Student's t-test	**<0.0001***

Supplemental Table 3: Cell Length

All cell length data were normally distributed as determined by Lilliefors' test. Significant values are bold and starred. Values with p>0.1 are written as NS (Not Significant).

Comparison	**Statistical Test**	***p*-value**
Subset: Isotropic Tissues	One-way ANOVA	NS
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	NS
Soft PDMS vs Gelatin	Fisher's LSD	<0.07
Subset: Aligned Tissues	One-way ANOVA	NS
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	<0.08
Soft PDMS vs Gelatin	Fisher's LSD	NS
Subset: Stiff PDMS, Isotropic vs Aligned	Student's t-test	**<0.0001***
Subset: Soft PDMS, Isotropic vs Aligned	Student's t-test	**<0.0003***
Subset: Gelatin, Isotropic vs Aligned	Student's t-test	**<0.0002***

Supplemental Table 4: Cell Width

All cell width data were normally distributed as determined by Lilliefors' test. Significant values are bold and starred. Values with p>0.1 are written as NS (Not Significant).

Comparison	**Statistical Test**	***p*-value**
Subset: Isotropic Tissues	One-way ANOVA	NS
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	NS
Soft PDMS vs Gelatin	Fisher's LSD	NS
Subset: Aligned Tissues	One-way ANOVA	<0.03
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	**<0.04***
Soft PDMS vs Gelatin	Fisher's LSD	**<0.02***
Subset: Stiff PDMS, Isotropic vs Aligned	Student's t-test	**<0.0001***
Subset: Soft PDMS, Isotropic vs Aligned	Student's t-test	**<0.0001***
Subset: Gelatin, Isotropic vs Aligned	Student's t-test	**<0.0001***

Supplemental Table 5: Cell Aspect Ratio

Cell aspect ratio data were not normally distributed as determined by Lilliefors' test. Significant values are bold and starred. Values with p>0.1 are written as NS (Not Significant).

Comparison	**Statistical Test**	***p*-value**
Subset: Isotropic Tissues	Kruskal-Wallis	NS
Stiff PDMS vs Soft PDMS	Fisher's LSD	**<0.05***
Stiff PDMS vs Gelatin	Fisher's LSD	NS
Soft PDMS vs Gelatin	Fisher's LSD	NS
Subset: Aligned Tissues	Kruskal-Wallis	<0.09
Stiff PDMS vs Soft PDMS	Fisher's LSD	<0.08
Stiff PDMS vs Gelatin	Fisher's LSD	NS
Soft PDMS vs Gelatin	Fisher's LSD	**<0.05***
Subset: Stiff PDMS, Isotropic vs Aligned	Student's t-test	**<0.0001***
Subset: Soft PDMS, Isotropic vs Aligned	Student's t-test	**<0.0001***
Subset: Gelatin, Isotropic vs Aligned	Student's t-test	**<0.0001***

Supplemental Table 6: Full FOV vs Uniaxial Conduction Velocity

Conduction velocity data comparing FOV choice were not normally distributed as determined by Lilliefors' test. Significant values are bold and starred. Values with p>0.1 are written as NS (Not Significant).

Comparison	**Statistical Test**	***p*-value**
Subset: Stiff PDMS, Isotropic	Student's t-test	NS
Subset: Stiff PDMS, Longitudinal	Student's t-test	**<0.04***
Subset: Stiff PDMS, Transverse	Student's t-test	NS
Subset: Soft PDMS, Isotropic	Student's t-test	NS
Subset: Soft PDMS, Longitudinal	Student's t-test	<0.09
Subset: Soft PDMS, Transverse	Student's t-test	NS
Subset: Gelatin, Isotropic	Student's t-test	NS
Subset: Gelatin, Longitudinal	Student's t-test	NS
Subset: Gelatin, Transverse	Student's t-test	NS

Supplemental Table 7: Uniaxial Conduction Velocity

Uniaxial conduction velocity data were not normally distributed as determined by Lilliefors' test. Significant values are bold and starred. Values with p>0.1 are written as NS (Not Significant).

Comparison	**Statistical Test**	***p*-value**
Subset: Isotropic Tissues	Kruskal-Wallis	NS
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	NS
Soft PDMS vs Gelatin	Fisher's LSD	NS
Subset: Aligned Tissues, Longitudinal	Kruskal-Wallis	NS
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	NS
Soft PDMS vs Gelatin	Fisher's LSD	NS
Subset: Aligned Tissues, Transverse	Kruskal-Wallis	<0.009
Stiff PDMS vs Soft PDMS	Fisher's LSD	NS
Stiff PDMS vs Gelatin	Fisher's LSD	**<0.04***
Soft PDMS vs Gelatin	Fisher's LSD	**<0.003***
Subset: Stiff PDMS	Kruskal-Wallis	<0.002
Isotropic vs Longitudinal	Fisher's LSD	NS
Isotropic vs Transverse	Fisher's LSD	**<0.003***
Longitudinal vs Transverse	Fisher's LSD	**<0.0005***
Subset: Soft PDMS	Kruskal-Wallis	<0.007
Isotropic vs Longitudinal	Fisher's LSD	NS
Isotropic vs Transverse	Fisher's LSD	**<0.02***
Longitudinal vs Transverse	Fisher's LSD	**<0.003***
Subset: Gelatin	Kruskal-Wallis	<0.0006
Isotropic vs Longitudinal	Fisher's LSD	NS
Isotropic vs Transverse	Fisher's LSD	**<0.002***
Longitudinal vs Transverse	Fisher's LSD	**<0.0003***

Supplemental Table 8: Uniaxial Conduction Velocity Anisotropy Ratio

All anisotropy ratio data were normally distributed as determined by Lilliefors' test. Significant values are bold and starred. Values with p>0.1 are written as NS (Not Significant).

Comparison	**Statistical Test**	***p*-value**
Subset: Aligned Tissues	One-way ANOVA	<0.002
Stiff PDMS vs Soft PDMS	Fisher's LSD	**<0.03***
Stiff PDMS vs Gelatin	Fisher's LSD	<0.07
Soft PDMS vs Gelatin	Fisher's LSD	**<0.0003***