**SUPPORTING INFORMATION:**

# Optimizing Blended Collagen-Fibrin Hydrogels for Cardiac Tissue Engineering with Human iPSC-derived Cardiomyocytes

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10 Pages, 7 Figures, 13 Tables

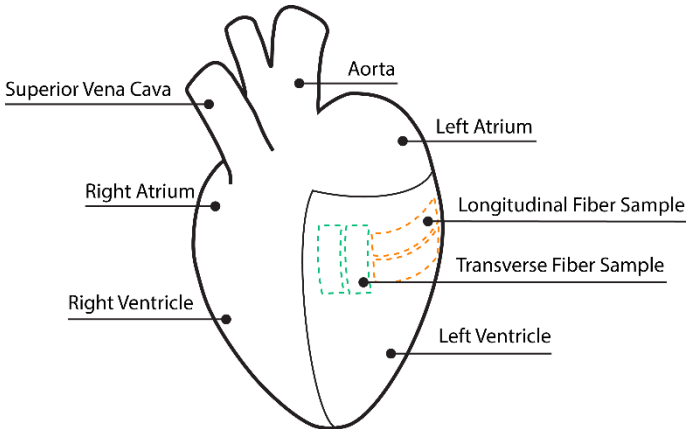


**Figure S1: Native rat myocardium sample collection.** Longitudinal fiber samples approximately 2 mm x 10 mm (orange) were collected from the left ventricle myocardial layer with the long axis aligned with cardiac muscle fibers. Conversely, transverse fiber samples with the same dimensions (green) were collected from the left ventricle myocardial layer with the long axis perpendicular to cardiac muscle fibers.

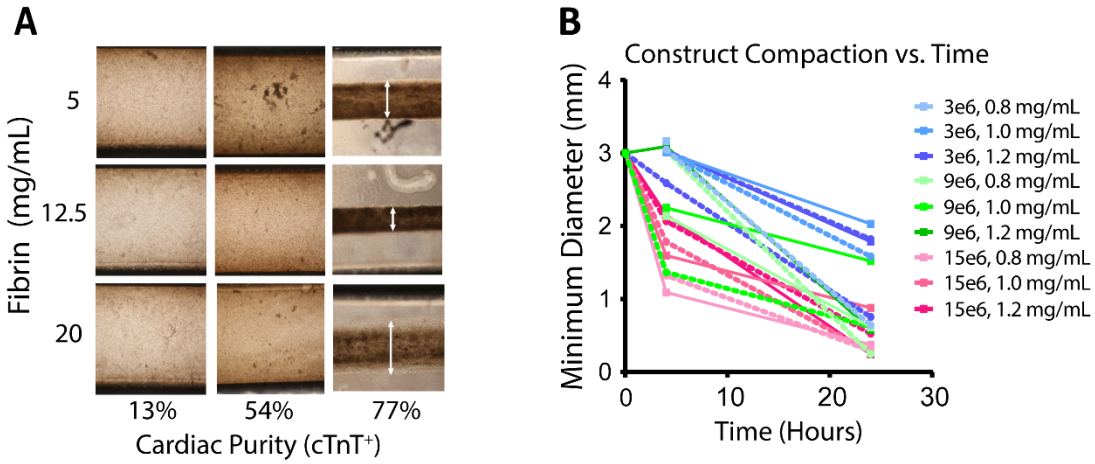


**Figure S2: Compaction of cellularized hydrogel constructs over time.** (A) Phase microscopy images of blended 2 mg/mL collagen and fibrin hydrogels after 144 hours of incubation under cell culture conditions. Fibrin concentration is indicated on the y-axis, and $cTnT^+$ percentage (a measure of hiPSC-derived cardiomyocyte purity) is indicated on the x-axis. Double headed arrows indicate the construct midpoint diameter that was measured. No fibrinolytic inhibitor was used in this single experiment. (B) Scatterplot of collagen-only 49.71% $cTnT^+$ cardiac construct diameter at the narrowest point is shown through culture time. Replicates prepared under the same conditions are paired by color and have solid and dashed lines. The legend indicates cell seeding density per mL and collagen concentration.

| Group | Collagen (mg/mL) | Fibrin (mg/mL) | Seeding Density ($1x10^6$ cells/mL) |
|---|---|---|---|
| 1 | 0.8 | 0 | 12 |
| 2 | 0.8 | 4 | 9 |
| 3 | 0.8 | 8 | 12 |
| 4 | 1.2 | 0 | 9 |
| 5 | 1.2 | 4 | 12 |
| 6 | 1.2 | 8 | 9 |
| 7 | 1.6 | 0 | 12 |
| 8 | 1.6 | 4 | 9 |
| 9 | 1.6 | 8 | 12 |
| 10 | 0.8 | 4 | 15 |
| 11 | 1.2 | 0 | 15 |
| 12 | 1.2 | 8 | 15 |
| 13 | 1.6 | 4 | 15 |

**Table S1: Response surface model groups.** Collagen, fibrin, and seeding density factor levels that defined each group. Note that group 5 was considered a center point for the Box-Behnken design format and was replicated across each of the two six well plates required for each experiment.

| Experiment | $cTnT^+$ (%) | $SMA^+$ (%) | $cTnT^+$ and $SMA^+$ (%) | $SMA^+$ only (%) |
|---|---|---|---|---|
| Unpurified 1 | 60.2 | 25.9 | 18.7 | 7.20 |
| Unpurified 2 | 24.4 | 51.2 | 8.11 | 43.1 |
| Unpurified 3 | 30.2 | 18.3 | 9.41 | 8.90 |
| Lactate Purified | 75.5 | 54.1 | 51.1 | 3.00 |
| GCaMP-CM | 52.2 | 25.8 | 7.90 | 17.9 |

**Table S2: Flow cytometry analysis of iPSC-derived cardiomyocytes.**

| | Force | | | | Calcium | | |
|---|---|---|---|---|---|---|---|
| Group | Active Stress (kPa) | $V_{up}$ ($mN/mm^2/s$) | $T_{50}$ (ms) | $T_{90}$ (ms) | $V_{up}$ (Intensity/s) | $T_{50}$ (ms) | $T_{90}$ (ms) |
| 4 | 0.02±0.01 | 0.08±0.04 | 131±24.0 | 237±26.7 | 11.5±2.99 | 118±66.0 | 195±84.7 |
| 11 | 0.08±0.01* | 0.42±0.07* | 96.7±3.18 | 193±8.39 | 8.44±2.00 | 86.0±16.3 | 159±38.9 |
| Predicted | 0.10 | 0.57 | 87.0 | 174 | 25.7 | 80 | 161 |

**Table S3: Force and calcium kinetics of constructs prepared with hiPSC-GCaMP-derived cardiomyocytes at 15% strain.** Only one replicate was evaluated for Group Predicted due to necking and breaking failure (n=3 for Groups 4 and 11). * indicates $p<0.05$ versus group 4.

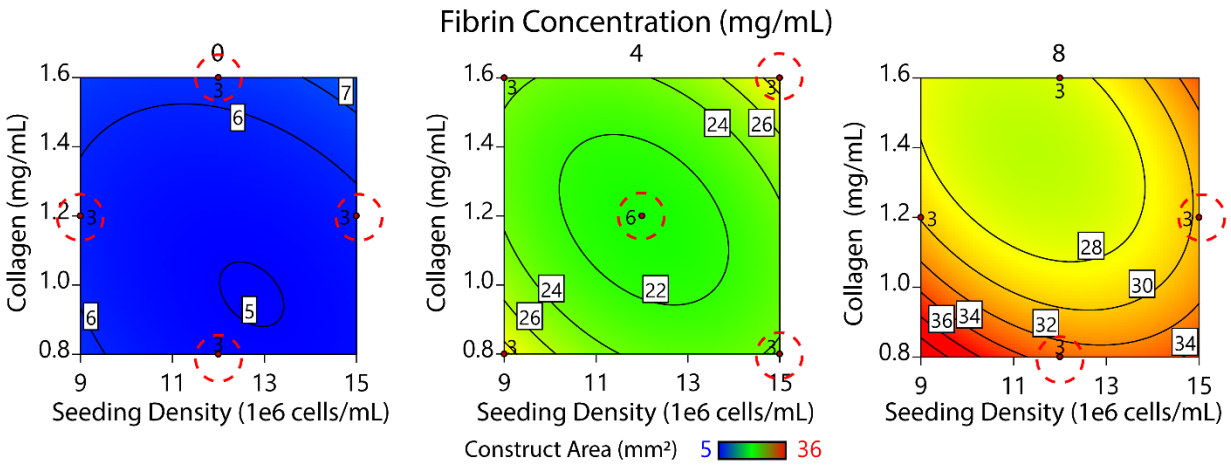


**Figure S3:** Contour plots describing predicted construct compaction with lactate purified cardiomyocytes (75% cTnT$^+$) after 72 hours of culture with respect to fibrin concentration (major x-axis), collagen concentration (minor y-axes), and seeding density (minor x-axes). Contour labels indicate the predicted construct two-dimensional area in mm$^2$ after 72 hours of culture. Red dots and adjacent numbers indicate the number of empirical data replicates for a corresponding factor set, as prescribed by the Box-Behnken design. Red dashed circles indicate uniformly beating groups.

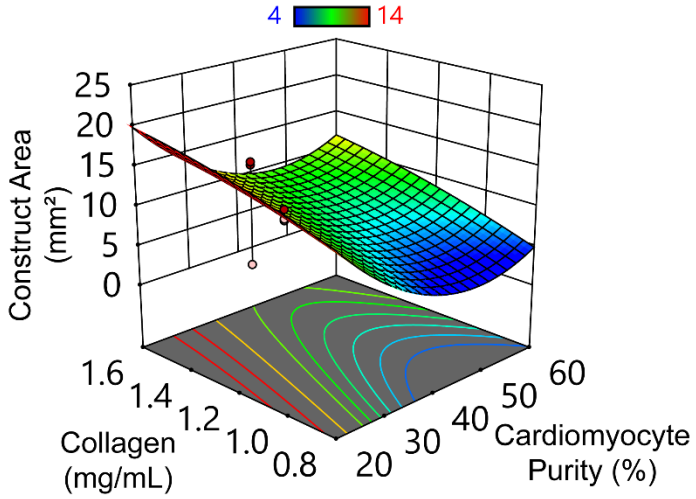


**Figure S4: Response surface model for unpurified construct compaction at 72 hours with 8 mg/mL fibrin.** Red dots indicate experimental sample value greater than the model prediction and pink dots indicate experimental sample values less than the model prediction. Cardiomyocyte purity is a defined as the percentage of cTnT$^+$ cells.

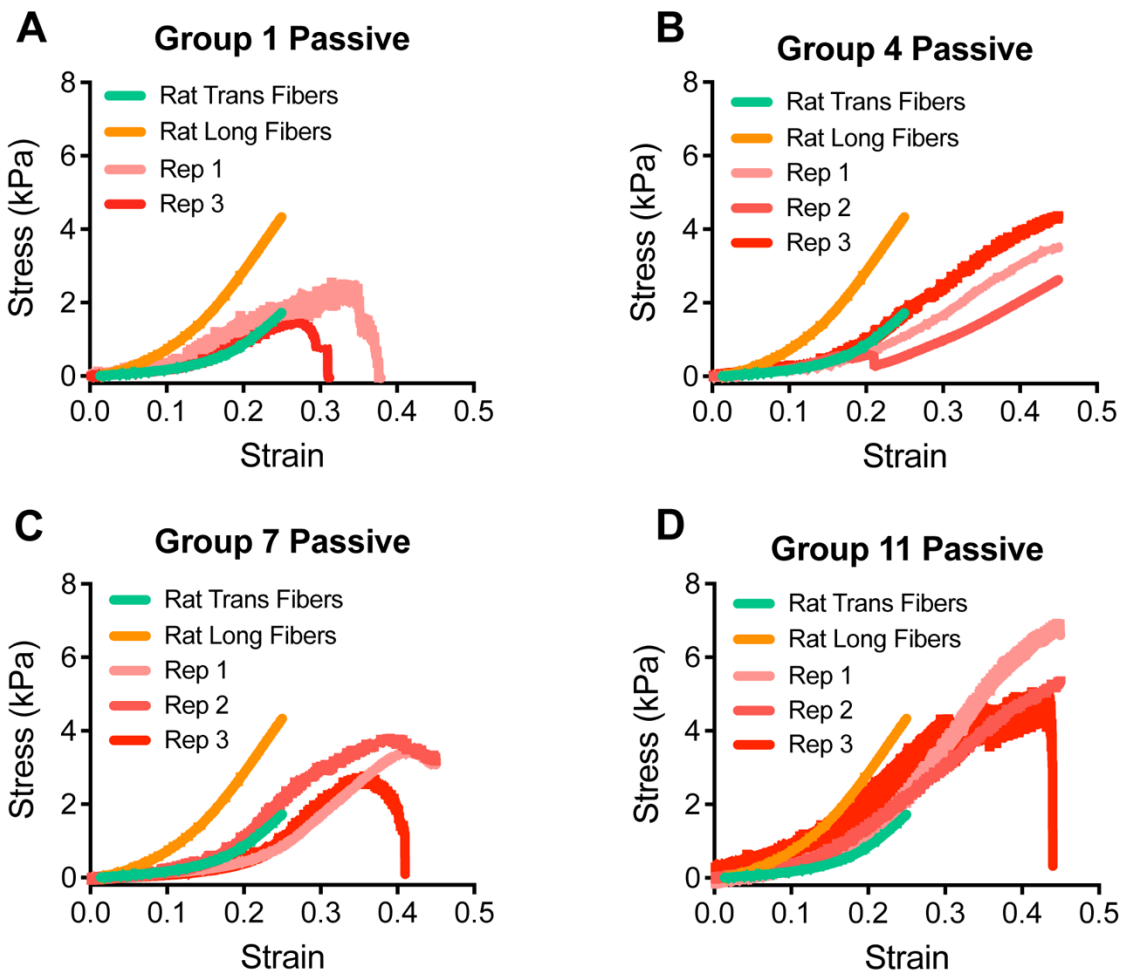


**Figure S5: Passive mechanical analysis of lactate purified constructs.** Green and orange traces represent stress/strain curves of native rat myocardium in the transverse and longitudinal fiber orientations (replicated from Figure 2C), respectively. Red shaded traces represent individual samples from Group 1 (A), Group 4 (B), Group 7 (C), and Group 11 (D). Increased "thickness" of some traces is a result of autonomous construct beating. All native and engineered tissues were tested at a constant strain rate of 10% strain/min.

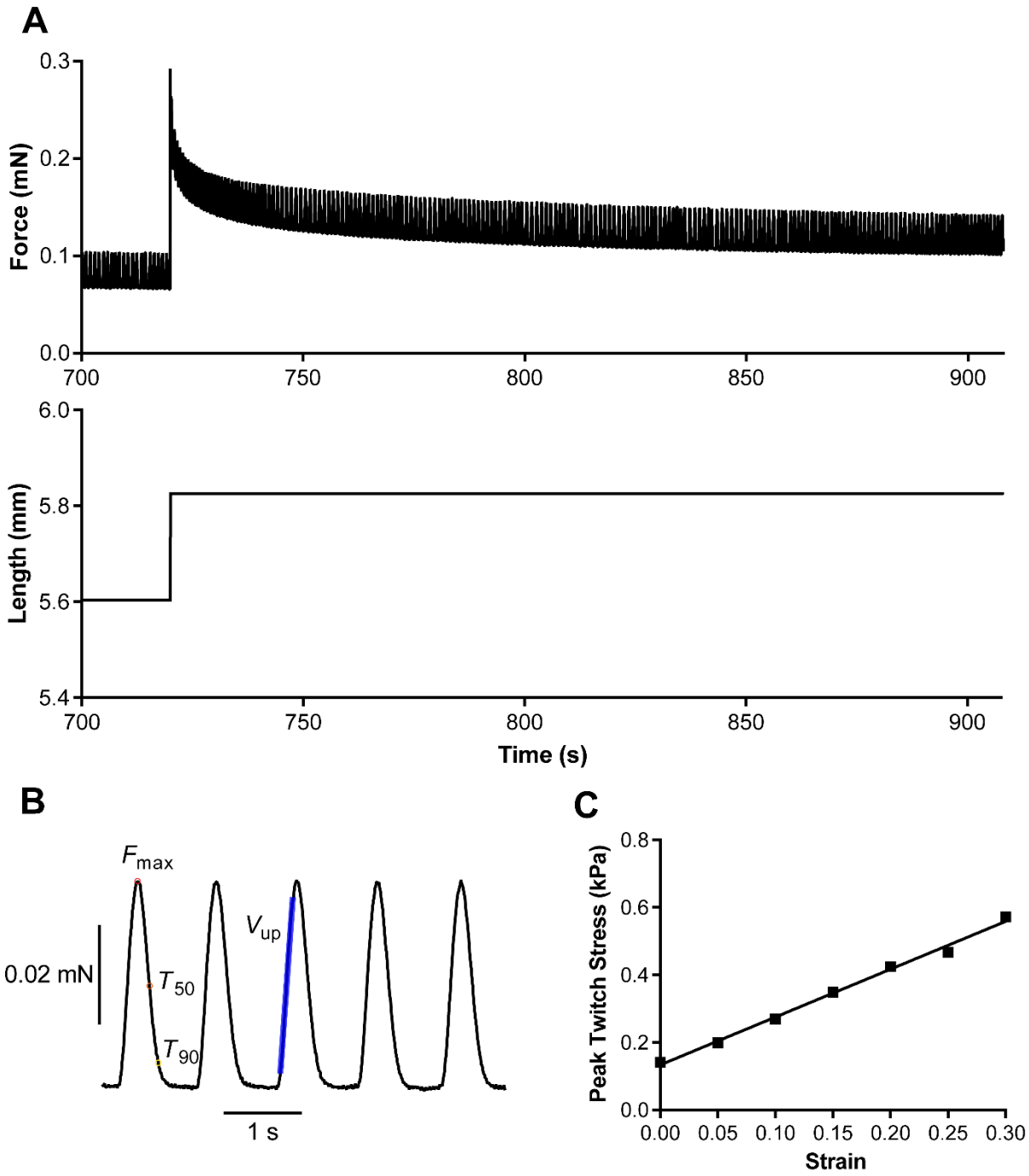


**Figure S6: Representative active mechanics analysis of lactate purified groups.** (A) Raw force and length vs time traces at maximum strain (30%) during 1 Hz stimulation. (B) Active contraction raw data trace shows peak force ($F_{max}$), which is normalized to cross-sectional area to calculate stress, upstroke velocity ($V_{up}$), and points used to calculate relaxation time to 50% and 90% relaxation ($T_{50}$ and $T_{90}$, respectively). Values are summarized in Table 3 at maximum (30%) strain. (C) Force-length relationship for one example tissue is shown as maximum twitch stress vs. strain at each of seven length positions ($R^2$ = 0.99).

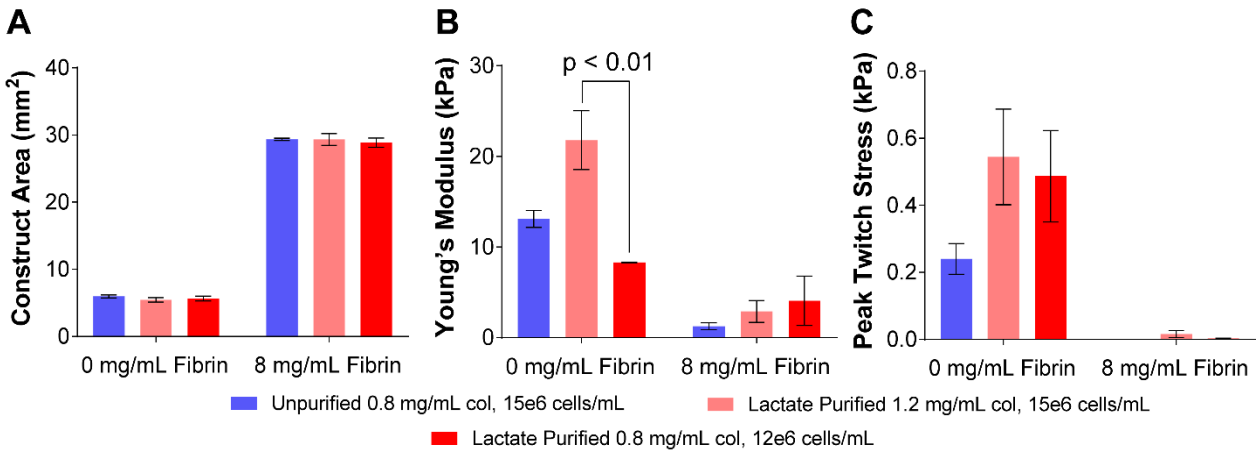


**Figure S7: Constructs prepared with high purity, unpurified iPSC-cardiomyocytes (84.6% cTnT$^+$) compared to select lactate purified cardiomyocyte (75.5% cTnT$^+$) groups.** Lactate purified Group 1, Group 3, Group 11, and Group 12 are described above by formulation factor levels to facilitate comparison. (A) Construct area after 72 hours in culture conditions. (B) Young's modulus and (C) Peak stress after 6 days in culture conditions.

| Source | Sum of squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 34.36 | 6 | 5.73 | 13.40 | < 0.0001 | significant |
| A-Fibrin | 3.84 | 1 | 3.84 | 8.99 | 0.0046 | |
| B-Collagen | 1.57 | 1 | 1.57 | 3.67 | 0.0624 | |
| D-Polymerization Temp | 2.28 | 1 | 2.28 | 5.34 | 0.0260 | |
| E-Time in Culture Conditions | 19.15 | 1 | 19.15 | 44.80 | < 0.0001 | |
| AE | 5.79 | 1 | 5.79 | 13.54 | 0.0007 | |
| DE | 1.73 | 1 | 1.73 | 4.05 | 0.0507 | |
| **Residual** | 17.52 | 41 | 0.4274 | | | |
| Lack of Fit | 2.96 | 9 | 0.3292 | 0.7234 | 0.6841 | not significant |
| Pure Error | 14.56 | 32 | 0.4550 | | | |
| **Cor Total** | 51.89 | 47 | | | | |

**Table S4: Acellular compression model fit.** df, degrees of freedom.

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High |
|---|---|---|---|---|---|
| Intercept | 1.42 | 1 | 0.0944 | 1.23 | 1.61 |
| A-Fibrin | 0.2830 | 1 | 0.0944 | 0.0924 | 0.4735 |
| B-Collagen | 0.1808 | 1 | 0.0944 | -0.0098 | 0.3714 |
| D-Polymerization Temp | 0.2180 | 1 | 0.0944 | 0.0275 | 0.4086 |
| E-Time in Culture Conditions | 0.6316 | 1 | 0.0944 | 0.4410 | 0.8222 |
| AE | 0.3472 | 1 | 0.0944 | 0.1567 | 0.5378 |
| DE | 0.1900 | 1 | 0.0944 | -0.0006 | 0.3806 |

**Table S5: Acellular compression model coefficients.**

| Source | Sum of squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 863.75 | 14 | 61.70 | 9.69 | < 0.0001 | significant |
| A-Cell Number | 29.85 | 1 | 29.85 | 4.69 | 0.0326 | |
| B-Collagen Concentration | 251.80 | 1 | 251.80 | 39.54 | < 0.0001 | |
| C-Fibrin Concentration | 80.62 | 1 | 80.62 | 12.66 | 0.0006 | |
| D-Cell Purity | 320.60 | 1 | 320.60 | 50.34 | < 0.0001 | |
| AB | 8.11 | 1 | 8.11 | 1.27 | 0.2616 | |
| AC | 5.10 | 1 | 5.10 | 0.8016 | 0.3726 | |
| AD | 25.60 | 1 | 25.60 | 4.02 | 0.0474 | |
| BC | 31.26 | 1 | 31.26 | 4.91 | 0.0288 | |
| BD | 1.20 | 1 | 1.20 | 0.1883 | 0.6652 | |
| CD | 1.79 | 1 | 1.79 | 0.2806 | 0.5974 | |
| $A^2$ | 67.26 | 1 | 67.26 | 10.56 | 0.0015 | |
| $B^2$ | 12.80 | 1 | 12.80 | 2.01 | 0.1591 | |
| $C^2$ | 20.99 | 1 | 20.99 | 3.30 | 0.0722 | |
| $D^2$ | 150.49 | 1 | 150.49 | 23.63 | < 0.0001 | |
| **Residual** | 687.75 | 108 | 6.37 | | | |
| Lack of Fit | 150.06 | 24 | 6.25 | 0.9768 | 0.5041 | not significant |
| Pure Error | 537.68 | 84 | 6.40 | | | |
| **Cor Total** | 1551.49 | 122 | | | | |

**Table S6: Unpurified 72 hour compaction model fit.**

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High |
|---|---|---|---|---|---|
| Intercept | 7.35 | 1 | 0.9650 | 5.43 | 9.26 |
| A-Cell Number | -0.6576 | 1 | 0.3037 | -1.26 | -0.0556 |
| B-Collagen Concentration | 1.92 | 1 | 0.3051 | 1.31 | 2.52 |
| C-Fibrin Concentration | -1.08 | 1 | 0.3049 | -1.69 | -0.4804 |
| D-Cell Purity | -3.18 | 1 | 0.4478 | -4.07 | -2.29 |
| AB | 0.4820 | 1 | 0.4271 | -0.3645 | 1.33 |
| AC | -0.3825 | 1 | 0.4272 | -1.23 | 0.4643 |
| AD | -0.7637 | 1 | 0.3809 | -1.52 | -0.0088 |
| BC | 0.9470 | 1 | 0.4274 | 0.0998 | 1.79 |
| BD | 0.1668 | 1 | 0.3845 | -0.5952 | 0.9289 |
| CD | -0.2045 | 1 | 0.3861 | -0.9699 | 0.5608 |
| $A^2$ | 1.54 | 1 | 0.4747 | 0.6018 | 2.48 |
| $B^2$ | -0.6732 | 1 | 0.4748 | -1.61 | 0.2680 |
| $C^2$ | 0.8620 | 1 | 0.4748 | -0.0791 | 1.80 |
| $D^2$ | 5.60 | 1 | 1.15 | 3.31 | 7.88 |

**Table S7: Unpurified 72 hour compaction model coefficients.**

| Source | Sum of squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 20.90 | 9 | 2.32 | 86.24 | < 0.0001 | significant |
| A-Cell Number | 0.0000 | 1 | 0.0000 | 0.0008 | 0.9779 | |
| B-Collagen Concentration | 0.0006 | 1 | 0.0006 | 0.0234 | 0.8794 | |
| C-Fibrin Concentration | 16.42 | 1 | 16.42 | 609.76 | < 0.0001 | |
| AB | 0.0798 | 1 | 0.0798 | 2.96 | 0.0948 | |
| AC | 0.0008 | 1 | 0.0008 | 0.0306 | 0.8621 | |
| BC | 0.1295 | 1 | 0.1295 | 4.81 | 0.0357 | |
| $A^2$ | 0.1260 | 1 | 0.1260 | 4.68 | 0.0381 | |
| $B^2$ | 0.1243 | 1 | 0.1243 | 4.62 | 0.0394 | |
| $C^2$ | 3.34 | 1 | 3.34 | 124.22 | < 0.0001 | |
| **Residual** | 0.8616 | 32 | 0.0269 | | | |
| Lack of Fit | 0.1652 | 3 | 0.0551 | 2.29 | 0.0988 | not significant |
| Pure Error | 0.6963 | 29 | 0.0240 | | | |
| **Cor Total** | 21.76 | 41 | | | | |

**Table S8: Lactate purified 72 hour compaction model fit.**

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High |
|---|---|---|---|---|---|
| Intercept | 3.04 | 1 | 0.0670 | 2.90 | 3.18 |
| A-Cell Number | -0.0009 | 1 | 0.0335 | -0.0692 | 0.0673 |
| B-Collagen Concentration | 0.0051 | 1 | 0.0335 | -0.0631 | 0.0733 |
| C-Fibrin Concentration | 0.8271 | 1 | 0.0335 | 0.7589 | 0.8953 |
| AB | 0.0815 | 1 | 0.0474 | -0.0149 | 0.1780 |
| AC | 0.0083 | 1 | 0.0474 | -0.0882 | 0.1048 |
| BC | -0.1039 | 1 | 0.0474 | -0.2004 | -0.0074 |
| $A^2$ | 0.1146 | 1 | 0.0530 | 0.0067 | 0.2224 |
| $B^2$ | 0.1138 | 1 | 0.0530 | 0.0059 | 0.2217 |
| $C^2$ | -0.5903 | 1 | 0.0530 | -0.6981 | -0.4824 |

**Table S8: Lactate purified 72 hour compaction model coefficients.**

| Source | Sum of squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 1206.24 | 3 | 402.08 | 15.76 | < 0.0001 | significant |
| A-Cell Number | 77.57 | 1 | 77.57 | 3.04 | 0.0895 | |
| B-Collagen Concentration | 409.18 | 1 | 409.18 | 16.04 | 0.0003 | |
| C-Fibrin Concentration | 670.54 | 1 | 670.54 | 26.29 | < 0.0001 | |
| **Residual** | 943.72 | 37 | 25.51 | | | |
| Lack of Fit | 361.74 | 9 | 40.19 | 1.93 | 0.0880 | not significant |
| Pure Error | 581.98 | 28 | 20.79 | | | |
| **Cor Total** | 2149.96 | 40 | | | | |

**Table S10: Lactate purified Young's modulus model fit.**

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High |
|---|---|---|---|---|---|
| Intercept | 8.60 | 1 | 0.7896 | 7.00 | 10.20 |
| A-Cell Number | 1.80 | 1 | 1.03 | -0.2910 | 3.89 |
| B-Collagen Concentration | 4.22 | 1 | 1.05 | 2.09 | 6.36 |
| C-Fibrin Concentration | -5.41 | 1 | 1.05 | -7.54 | -3.27 |

**Table S11: Lactate purified Young's modulus model coefficients.**

| Source | Sum of squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 73.44 | 6 | 12.24 | 30.85 | < 0.0001 | significant |
| A-Cell Number | 5.39 | 1 | 5.39 | 13.60 | 0.0008 | |
| B-Collagen Concentration | 0.6841 | 1 | 0.6841 | 1.72 | 0.1977 | |
| C-Fibrin Concentration | 49.49 | 1 | 49.49 | 124.73 | < 0.0001 | |
| AC | 0.6395 | 1 | 0.6395 | 1.61 | 0.2126 | |
| BC | 0.9568 | 1 | 0.9568 | 2.41 | 0.1295 | |
| $C^2$ | 16.27 | 1 | 16.27 | 41.01 | < 0.0001 | |
| **Residual** | 13.89 | 35 | 0.3968 | | | |
| Lack of Fit | 2.16 | 6 | 0.3597 | 0.8893 | 0.5154 | not significant |
| Pure Error | 11.73 | 29 | 0.4045 | | | |
| **Cor Total** | 87.33 | 41 | | | | |

**Table S12: Lactate purified peak active stress model fit.**

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High |
|---|---|---|---|---|---|
| Intercept | -4.18 | 1 | 0.1485 | -4.49 | -3.88 |
| A-Cell Number | 0.4741 | 1 | 0.1286 | 0.2131 | 0.7352 |
| B-Collagen Concentration | -0.1688 | 1 | 0.1286 | -0.4299 | 0.0922 |
| C-Fibrin Concentration | -1.44 | 1 | 0.1286 | -1.70 | -1.18 |
| AC | -0.2308 | 1 | 0.1818 | -0.6000 | 0.1383 |
| BC | 0.2824 | 1 | 0.1818 | -0.0868 | 0.6515 |
| $C^2$ | 1.26 | 1 | 0.1964 | 0.8590 | 1.66 |

**Table S13: Lactate purified peak active stress model coefficients.**