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Self-assembled smooth muscle cell tissue rings exhibit greater tensile strength than cell-seeded fibrin or collagen gel rings

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Abstract

In this study, we created self-assembled smooth muscle cell (SMC) tissue rings (comprised entirely of cells and cell-derived matrix; CDM) and compared their structure and material properties with tissue rings created from SMC-seeded fibrin or collagen gels. All tissue rings were cultured statically for 7 days in supplemented growth medium (with ϵ -amino caproic acid, ascorbic acid, and insulin-transferrin-selenium), prior to uniaxial tensile testing and histology. Self-assembled CDM rings exhibited ultimate tensile strength and stiffness values that were two-fold higher than fibrin gel and collagen gel rings. Tensile testing of CDM, fibrin gel and collagen gel rings treated with deionized water to lyse cells showed little to no change in mechanical properties relative to untreated ring samples, indicating that the ECM dominates the measured ring mechanics. In addition, CDM rings cultured in supplemented growth medium were significantly stronger than CDM rings cultured in standard, unsupplemented growth medium. These results illustrate the potential utility of self-assembled cell rings as model CDM constructs for tissue engineering and biomechanical analysis of ECM material properties.

Keywords

fibrin gel; collagen gel; cell-derived matrix; vascular tissue engineering; soft tissue biomechanics

Introduction

The motivation for the present study was to evaluate the structure and mechanical properties of engineered tissues created entirely from self-assembled cells and cell-derived matrix (CDM), compared to cell-populated fibrin or collagen biopolymer gels (the gold standard for fabricating totally biological tissue constructs). Due to their inherent biocompatibility, short time frame for forming three-dimensional (3D) constructs, and the extent to which cells can attach to and remodel these materials, fibrin and collagen gels are among the most versatile and widely utilized biomaterials in vascular tissue engineering (reviewed in ¹). Furthermore, collagen and fibrin gel constructs are relatively easy to fabricate; cells in suspension can be mixed with biopolymer gel materials in their liquid state, poured into a mold, and allowed to solidify, thus instantly creating a tubular tissue construct. However, despite their utility for creating 3D blood vessel equivalents *in vitro*, the use of biopolymer gels as scaffolds in transplantable vascular grafts has been limited to venous transplant models (e.g., ovine jugular vein²) due to their relatively poor mechanical strength without extensive culture or mechanical conditioning.^{3–6}

Cellular self-assembly without exogenous scaffold materials is an attractive alternative approach, which allows engineered vascular tissue fabrication entirely from cells and CDM. In our previous work, we developed a cellular self-assembly method to create ring-shaped tissue constructs and showed that they can fuse laterally to form tubular tissues.^{7,8} Other reported cellular self-assembly techniques include culturing cells as sheets and wrapping these sheets around a stainless steel mandrel⁹, or bioprinting¹⁰ and fusion^{10,11} of aggregated cell spheres. Autologous vascular grafts created with the “cell sheet”-based approach (also known as “tissue-engineering by self-assembly” or TESA; recently reviewed in¹²) have already been applied successfully in clinical trials as arterovenous fistulas.^{13,14} Although many cellular self-assembly methods are more time and resource intensive than creating tissue from cells in gels, CDM constructs have exhibited several potential advantages over scaffold-dependent tissue constructs, including improved strength and biocompatibility, greater cellularity, increased extracellular matrix (ECM) synthesis, and the ability to create tissues from human cell-derived materials.^{15,16}

In the present study, we compared the material properties of CDM rings and rings fabricated from an equivalent number of cells seeded in fibrin or collagen gels, to assess their relative strength and utility for tissue engineering. The growth medium used to culture CDM, fibrin gel and collagen gel constructs varies in the literature; therefore, all samples were cultured in the same supplemented growth medium containing insulin-transferrin-selenium (ITS), ascorbic acid, and ϵ -amino caproic acid. To ensure that the supplements did not adversely impact cellular self-assembly and CDM ring formation, we evaluated the effects of standard or supplemented growth medium on CDM ring structure and material properties. Tissue growth, structure, and biomechanical properties in CDM rings, and fibrin gel and collagen gel ring samples, were analyzed by uniaxial tensile testing and histology after 7 days in static culture. To evaluate the relative contributions of cell and ECM mechanics to tissue ring material properties, we also performed mechanical tests of CDM, fibrin gel and collagen gel rings with or without cell lysis.

Materials and Methods

Custom cell culture well fabrication

Custom ring-shaped agarose wells were created as previously described.^{7,8} Briefly, a polycarbonate mold was machined with ring-shaped wells with 4 mm diameter posts. Polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning, Midland, MI) was then cured onto the polycarbonate mold to create a negative template. Two percent agarose (w/v; Lonza, Rockland, ME) dissolved in Dulbecco's Modified Eagle Medium (DMEM; Mediatech, Herndon, VA) was autoclaved, poured onto the PDMS template, and cut into individual wells for cell seeding. Agarose wells were placed into 6-well plates and equilibrated in either standard growth medium or supplemented growth medium (as defined below) at 37°C and 5% CO₂ for 1 hour prior to cell seeding.

Smooth muscle cell culture and growth medium (standard v. supplemented)

Rat aortic smooth muscle cells (SMCs; WKY 3M-22 isolated from adult Wistar-Kyoto rat aortas^{17,18}; generously provided by Dr. Thomas Wight, Benaroya Research Institute, Seattle, WA) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; PAA, Ontario, Canada), penicillin/streptomycin, glutamine, sodium pyruvate, and non-essential amino acids (cell culture reagents purchased from Mediatech, Herndon, VA). This growth medium is referred to in the text as “standard” growth medium. “Supplemented” growth medium refers to standard growth medium supplemented with 1% insulin-transferrin-selenium (ITS, Invitrogen), 15 μ g/mL ascorbic acid (Sigma-Aldrich) and 1 mg/mL ϵ -amino caproic acid (Sigma).

Cell seeding for tissue ring formation

To ensure that supplemented growth medium did not adversely affect cellular self-assembly and tissue ring formation, CDM tissue rings were prepared in agarose wells pre-equilibrated with either standard or supplemented growth medium. To create CDM rings, SMCs were trypsinized, resuspended at a density of 5×10^6 cells/ml, and 150 μ L of cell suspension (750,000 cells) was seeded into each agarose well. Plates containing seeded wells were then placed in the incubator and cultured undisturbed for 2 days. Growth medium (standard or supplemented) was exchanged every 2 days thereafter for the total culture duration of 8 days.

SMCs settled in the wells, aggregated and contracted around the center posts to form cohesive tissue rings (Figure 1A). At the end of the culture period, CDM rings were removed from the wells (Figure 1B) and subjected to uniaxial tensile testing (Figure 1C).

To compare the structure and strength of CDM rings to tissue rings made from cell-seeded fibrin or collagen gels, SMCs were trypsinized and seeded directly into agarose wells, or an equivalent number of cells (750,000 cells in 150 μ L) was resuspended in fibrin or collagen gel and pipetted into agarose wells.

For the fibrin gel rings, 7.5mL of Hank's Buffered Saline Solution (HBSS; 20mM HEPES in 0.9% saline solution) was mixed with 1.5mL fibrinogen (final concentration: 3.8mg/ml; Sigma), and 2mL of cell suspension. In a separate tube, 2mL of DMEM was mixed with 7.5 μ L of 2N Ca^{++} (CaCl_2) and 100 μ L of thrombin (final concentration: 0.19U/ml; Sigma). These two solutions were mixed, pipetted into agarose wells equilibrated with supplemented growth medium (750,000 cells in 150 μ L per well), and transferred to an incubator at 37°C and 5% CO_2 .

For the collagen gel rings, 420 μ L of supplemented growth medium and cell solution was mixed with 300 μ L FBS (PAA), 480 μ L 5X DMEM (Mediatech, Herndon, VA), 300 μ L 0.1N NaOH, and 1500 μ L bovine type I collagen (dissolved in 0.02N acetic acid; final concentration: 2mg/ml; MP Biologicals). Collagen gel solution (750,000 cells in 150 μ L per well) was pipetted into agarose wells equilibrated with supplemented growth medium and transferred to an incubator at 37°C and 5% CO_2 .

Tissue rings (CDM, fibrin and collagen) were left undisturbed for an initial period of 2 days, after which the supplemented medium was changed every 2 days for the 7-day total culture duration.

Tissue ring thickness measurements

On the final day of each study, the tissue rings were removed from their agarose wells and placed in phosphate-buffered saline (PBS) under a machine vision system (DVT Model 630; DVT Corporation, Atlanta, GA). Tissue ring thicknesses were measured at four separate positions along the circumference of each ring, and thickness values for each treatment group were reported as mean $\pm$ S.E.M. The average thickness value for each ring was used to calculate cross-sectional area (assuming a circular cross-section, $A = \pi r^2$, where $r = \frac{1}{2}$ thickness and the total cross-sectional area is $2 \times A$ to account for two sides of the ring) as previously described.⁸

Mechanical testing

Tissue rings were mechanically tested using a uniaxial tensile testing machine (ElectroPuls E1000; Instron, Norwood, MA) as previously described.^{7,8} Briefly, each tissue ring was mounted on two small stainless steel pins (referred to as "grips"; shown in Figure 1C), and

submerged in PBS. The ring was then pulled to a tare load of 5mN and its gauge length (L_g) was recorded. Each ring was pre-cycled 8 times from the initial tare load to 50kPa engineering stress (F/A ; where A is calculated from the cross-sectional area), and then pulled to failure at a rate of 10 mm/min. The test protocol was programmed to terminate if the force transducer sensed a drop in measured force of 40% within 0.1 seconds (BlueHill Software), which correlated with complete CDM ring failure in previous studies.⁸

Using MATLAB (The MathWorks, Inc., Natick, MA), the engineering stress and grip-to-grip displacement data were analyzed to obtain the ultimate tensile strength (UTS), failure strain, and maximum tangent modulus (MTM, the maximum slope of the stress-strain curve; stiffness). These values were used for statistical comparisons of mechanical properties between each tissue ring type.

Isolation of matrix contributions to tissue ring mechanical properties

To evaluate the relative contributions of cells and ECM to tissue ring biomechanical properties, a separate batch of CDM, fibrin gel and collagen gel rings were prepared and cultured as described above. After 7 days, tissue rings were incubated in sterile deionized water (before removal from the agarose wells) for one hour to lyse the cells and eliminate cellular contraction.¹⁹ Tissue rings were re-equilibrated in isotonic PBS before lysed and untreated rings were subjected to thickness measurements and uniaxial testing as described above. For all samples in this experiment, the pull-to-failure test termination threshold was set to a value of 60% load reduction within 0.1 seconds.

Histology

Untested rings were fixed in 10% neutral-buffered formalin and embedded in paraffin. Five micrometer sections were cut, adhered to Superfrost Plus slides (VWR, West Chester, PA), and stained with hematoxylin and eosin (H&E; reagents from Richard Allan Scientific, Kalamazoo, MI) and Fast Green/Picrosirius Red (reagents from Sigma; 0.1% each of Fast Green FCF and Direct Red 80 in Picric Acid). Fast Green/Picrosirius Red stains collagen red and counterstains other tissue components green to allow visualization of interstitial collagen.²⁰ Microscopic images were acquired with a digital camera (Leica DFC 480) on an upright microscope (Leica DMLB2).

Statistics

A Student's t-test was used to analyze the effects of standard vs. supplemented medium on the thickness and mechanical properties of the rings ($n = 5$ and $n = 4$ for the standard and supplemented groups, respectively). A one-way ANOVA with Holm-Sidak post hoc analysis was used to compare the thicknesses and mechanical properties of the CDM, fibrin and collagen gel rings ($n = 6$ for each group). A two-way ANOVA with Holm-Sidak post hoc analysis was used to compare untreated or lysed CDM, fibrin gel and collagen gel rings ($n = 4$ per group). SigmaPlot software (Version 11.0 Systat Software, Inc.) was used to perform the statistical analyses and identify significant differences ($p < 0.05$) between parameter values.

Results

SMCs self-assembled and formed CDM tissue rings in supplemented growth medium

Prior to comparing CDM rings to fibrin gel and collagen gel rings, we first assessed the effects of medium supplementation on cellular self-assembly and ring formation of CDM constructs compared to CDM rings cultured in the "standard" growth medium. In both the supplemented and standard medium groups, SMCs consistently aggregated and contracted around the center posts of the agarose wells and successfully formed tissue rings within one

day of seeding, thereby demonstrating that the added supplements did not adversely affect SMC aggregation or CDM tissue ring formation. Therefore, supplemented growth medium was used for subsequent experiments with CDM, fibrin gel and collagen gel tissue rings.

CDM rings cultured in supplemented medium were mechanically stronger than control CDM rings cultured in standard growth medium

After 8 days, CDM rings cultured in standard growth medium were significantly thicker than CDM rings cultured in supplemented medium (0.63 ± 0.03 v. 0.54 ± 0.04 mm, respectively, $p < 0.05$; Figure 2A). CDM rings cultured with supplemented medium exhibited significantly higher ultimate tensile stress (Figure 2B) and MTM (Figure 2C) than CDM rings cultured in standard medium (UTS = 387 ± 48 v. 121 ± 41 kPa, $p < 0.05$, and MTM = 1593 ± 157 v. 662 ± 71 kPa, $p < 0.05$, respectively). There was not a statistically significant difference in failure strain between the two groups (Figure 2D). Both CDM rings cultured in standard and supplemented growth medium exhibited uniform cell distribution and high cell density across the tissue ring wall (Figure 3A, B), with interstitial collagen synthesis throughout (red staining; Figure 3C, D). Overall, there were no striking differences in tissue organization or morphology between CDM rings cultured in standard or supplemented growth medium.

Successful formation of CDM, fibrin and collagen gel tissue rings seeded in agarose wells

Consistent with results of the standard v. supplemented medium study, SMCs in the CDM tissue samples aggregated around the center posts of the agarose wells and formed rings within one day of cell seeding. Cell-loaded fibrin and collagen gels solidified and formed visible, semi-translucent rings around the center posts within 30 minutes of seeding. After four days in culture, the fibrin and collagen gel rings appeared less translucent and closely resembled the CDM rings by gross examination. After 7 days in culture, ring samples from all three experimental groups were easily removed from the wells for mechanical and histological analysis.

Fibrin gel rings were thicker than collagen gel rings and CDM rings after 7 days in culture

SMC-populated fibrin gel rings were significantly thicker than the CDM tissue rings and collagen gel rings, with an average thickness of 0.88 mm (58% thicker than CDM rings, and 41% thicker than collagen gel rings; $p < 0.05$) as shown in Figure 4A. However, there were no statistically significant differences between the mean thicknesses of the CDM and collagen gel tissue rings.

Differences in stress-strain curves and mechanical failure properties were observed between CDM, fibrin and collagen gel ring samples

The failure profile of each ring type varied as demonstrated by example stress-strain curves for each experimental ring type shown in Figure 5. The CDM and collagen gel tissue rings behaved like typical biological tissues during uniaxial tensile testing in terms of failure behavior.²¹ CDM and collagen gel rings consistently broke completely at a discrete failure point between the grips, which was confirmed both visually and from the plotted stress-strain data. In contrast, fibrin gel tissue rings displayed a “fraying” effect during failure. Unlike the CDM tissue rings, fibrin gel tissue rings did not break at a discrete point across the entire thickness of the ring; but rather appeared to fail in distinct intermediate steps (Figure 5). This resulted in variable pull-to-failure test outcomes for the fibrin gel rings depending on the magnitude of the first observed failure point in the ring. Half of the fibrin gel samples in this experiment had a complete pull-to-failure test and a complete ring break at failure (as shown in Figure 5). In the other samples, the first failure point resulted in a sudden sharp reduction in load which was detected as a catastrophic failure and triggered

termination of the test. However, one or more thin “strands” of the ring were usually still intact. For the computational analysis, the point at which the test was terminated was evaluated as the failure point.

CDM tissue rings were stronger and stiffer than biopolymer gel tissue rings

After 7 days of culture, the CDM tissue rings displayed significantly higher UTS than the fibrin gel and collagen gel tissue rings (298 ± 71 , 152 ± 37 , and 117 ± 20 kPa, respectively; $p < 0.05$, Figure 4B). Likewise, the CDM tissue rings also displayed significantly higher MTM than the fibrin and collagen gel tissue rings (559 ± 91 , 221 ± 84 , and 324 ± 25 kPa, respectively; $p < 0.05$, Figure 4C). There were no statistically significant differences between the CDM rings and the fibrin gel and collagen gels with respect to failure strain ($p < 0.05$; Figure 4D).

Differences in structure and morphology of CDM, fibrin gel, and collagen gel tissue rings

To evaluate the cellular organization and tissue architecture within tissue rings from each experimental group, rings were harvested at 7 days and fixed and processed for histology. Representative micrographs of each untested ring type are shown in Figure 6. In the CDM rings, the cells appeared more aligned at the inner and outer edges of the rings (Figure 6A); and the cell density appeared uniform throughout the tissue. In the fibrin gel and collagen gel rings, the cell density appeared to be greater at the outer edges of the tissue (Figure 6B, C). Analysis of the rings stained with Fast Green/Picrosirius Red showed that within the CDM tissue rings, collagen (red staining, Figure 6D) appeared to be evenly distributed throughout the tissue. In contrast, the fibrin gel and collagen gel rings were organized in distinct stratified layers of cells and collagen (Figure 6E, F). The appearance of distinct cell layers during collagen and fibrin gel remodeling is consistent with data reported in other studies.^{3,22}

Cells are uniformly distributed throughout fibrin and collagen gels initially, but remodel into stratified cellular layers with time in culture

The histological analysis and mechanical failure modes of each ring type revealed distinct layers of cells and matrix within fibrin gel and collagen gel rings which was not observed in the CDM rings. To confirm that cells were evenly distributed within the fibrin and collagen gels initially, cell-populated fibrin and collagen gels were seeded and examined histologically at earlier time points (1, 2, and 3 days) after ring seeding. Cells in both the fibrin (Figure 7 A–C) and collagen gel (Figure 7 D–F) rings were uniformly distributed throughout the tissue at one and two days after seeding. However, at the three day time point, cells in the collagen gels appeared to re-distribute toward the outer edges of the ring, forming stratified layers of cells (Figure 7F), similar to those observed by histology after 7 days (Figure 6F).

Tissue ring mechanical properties are contributed primarily by the ECM

To determine the extent to which cells and ECM (or both) contributed to tissue ring mechanics, we conducted an additional experiment in which tissue rings (CDM, fibrin gel or collagen gel) were pre-treated with deionized water to kill the cells and eliminate active cellular contraction.¹⁹ In general, cell lysis had little to no effect on tissue ring thickness, UTS, MTM or failure strain (Figure 8). Small but statistically significant differences were observed in collagen gel ring thickness (~16% greater with deionized water treatment; Figure 8A), and CDM ring UTS and MTM (each ~9% greater in untreated samples; Figure 8B, C). A larger difference was observed in collagen gel ring failure strain values with or without cell lysis (34% greater in untreated v. lysed samples; Figure 8D). Overall, the results of this experiment were consistent with those reported in the previous experiment (Figure 4),

with CDM tissue rings exhibiting ~ 2-fold greater UTS and MTM than fibrin or collagen gel rings and no statistically significant differences in failure strain values between CDM, fibrin gel and collagen gel rings. However, the magnitude of the UTS and MTM values were ~ 2-fold greater overall, and collagen gel ring thickness values were greater than in the previous experiment ($0.76 \pm 0.06 \text{ mm}$ v. $0.62 \pm 0.03 \text{ mm}$).

All fibrin gel ring samples (4/4 of the untreated and lysed samples) in this experiment exhibited the same step-wise decreases in load during failure, visible “fraying” of the rings, sharp decrease in load and termination of the pull-to-failure test when the rings were nearly (but not completely) broken, which was consistent with the results of the previous experiment.

Discussion

The overall goal of this study was to compare the material properties of tissue ring constructs made from smooth muscle cells seeded in fibrin and collagen gels with those of tissue rings made from an equivalent number of smooth muscle cells and CDM. There is increasing interest in CDM as a biomaterial given recent clinical²³ and pre-clinical^{24,25} evidence that devitalized tissue engineered blood vessels (TEBV) comprised from CDM alone may prove effective as “off-the-shelf” small diameter vascular grafts. However, few studies have directly compared the biomechanics of CDM with those of scaffold-based constructs such as cell-populated gels, and the length of time typically required to synthesize model CDM tissue constructs for mechanical testing has limited systematic analysis of CDM material properties. In this study, we describe a method to create model CDM tissue rings within one week that are stronger than tissue rings made from an equivalent number of cells seeded in fibrin or collagen gel.

A number of factors can affect the mechanical properties of engineered tissues, including media supplementation, mechanical conditioning, cell source, cell number and biopolymer (fibrinogen or type I collagen) type and concentration. For example, Grassl et al. showed that cells in fibrin gels produce almost five times more collagen than cells in collagen gels, which corresponded with increased mechanical strength in fibrin gel constructs.²⁶ In this study, we used standard cell-populated fibrin and collagen gel fabrication methods and protein concentrations described in the literature^{6,19,26}, while keeping the initial cell number and culture medium components constant for all three tissue types (CDM, fibrin gel and collagen gel). Supplements were used primarily for the purpose of normalizing culture conditions across CDM, fibrin gel and collagen gel samples. In particular, we included ϵ -amino caproic acid because cell-populated fibrin gels have been shown to degrade without protease inhibition²⁶, and we were concerned that the mechanical integrity of the fibrin gel constructs would be disproportionately affected. ITS and ascorbic acid have been shown to improve engineered tissue strength in a number of studies by stimulating ECM synthesis. Ascorbic acid is commonly included in growth medium to promote collagen synthesis in all three types of tissue constructs^{9,26}, and insulin has been shown to increase strength and elastin synthesis in some vascular tissue engineering studies.²⁷ Although ECM was not quantified in the present study, we observed a three-fold increase in tensile strength and stiffness in CDM rings cultured in supplemented (v. standard) growth medium. This suggests that the cell ring self-assembly system may provide a useful tool for quantifying the functional effects of specific chemical factors on cell-derived ECM biomechanics in future studies.

The methods used for testing and analysis of soft tissue constructs can also impact the outcome of biomechanical experiments. The methods used in this study were developed based on values obtained from published reports, and preliminary testing of CDM ring

samples. We chose to pre-cycle the rings for consistency with accepted practices for obtaining reproducible stress-strain curves during uniaxial tensile testing²¹, and chose to measure UTS, MTM and failure strain because these are the most common metrics reported in the biomechanics literature.

In general, the biomechanical strength of the CDM, fibrin gel, and collagen gel rings reported here were greater than those reported for cell-populated gel constructs cultured statically for a similar duration in other studies. For example, the average UTS values of the collagen gel rings (~120–140kPa) was higher than values reported for collagen gel tissue constructs made with rat aortic SMCs cultured statically (36.1kPa at 6 days²⁸, 16kPa at 8 days⁶). The average UTS values for fibrin gel rings in our studies (150–270kPa) were also higher than fibrin gel tissue constructs described in other studies (15.6kPa at 6 days²⁸). The MTM of our collagen and fibrin gel tissues (300–370kPa and 220–660kPa, respectively) also exceeded those of comparable tissue constructs (191.4kPa and 27.9kPa for collagen and fibrin, respectively²⁸). For perspective, although the strength of our CDM tissues (300–650kPa) was also much higher than previously reported values for fibrin and collagen gel constructs, these values are relatively low compared to native arteries (e.g., porcine carotid artery UTS ~6.6MPa²⁹; rat abdominal aorta, 2.1MPa³⁰). In future studies, it may be possible to further strengthen CDM tissue rings by mechanical conditioning^{5,6} and additional media supplements.¹⁶

Although the number of cells per ring used was lower than the cell number used in our previously work (750,000 v. 1.3 million cells per 4mm ring)⁸, the cell density is much higher (five million cells/mL) compared to other cell-populated gel studies (e.g., one million cells/mL²⁸). The cell seeding density was determined empirically as the critical minimum number of cells required to consistently achieve SMC aggregation and CDM ring formation, and was used consistently for CDM, fibrin gel and collagen gel rings to minimize differences between sample groups. Tensile strength in engineered tissues also depends on the cell source, and differences in the quantity, composition and organization of the ECM synthesized. For example, dermal fibroblasts produce greater amounts of collagen than smooth muscle cells, which results in greater tensile strength in fibroblast-based CDM constructs.³¹

Herein, we created tissue ring constructs (4mm diameter) from cells in fibrin gel, collagen gel, and CDM and found that ring constructs made from aggregated cells and CDM were reproducibly stronger than constructs made from cells in fibrin or collagen gels. These results are consistent with the finding that planar human dermal fibroblast CDM tissue constructs were stronger than planar fibroblast-populated fibrin and collagen gel constructs (after three weeks in culture).¹⁶ Tissue ring mechanical properties were not affected by cell lysis, which indicates that ring biomechanics are due to ECM properties. The cellular self-assembly system used to create CDM rings may therefore offer a feasible alternative to cell-seeded gels as a means of rapidly fabricating model engineered tissues for quantitative functional assessment of CDM mechanics. In addition, a long term goal of our CDM ring studies is to systematically screen and optimize conditions (e.g., cell source, culture supplements, mechanical stimulation parameters) that impact cell-mediated ECM synthesis to develop tunable engineered tissues. In our previous work, we showed that CDM rings fuse laterally to create tube-shaped tissue constructs^{7,8}, therefore the results of CDM ring biomechanics studies may be applied to the construction of tissue engineered blood vessels.

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Figure 1. Culture and mechanical testing of self-assembled CDM rings

Photographs of a 4 mm ID self-assembled smooth muscle cell ring in an agarose well after 7 days in culture (A), in saline in a Petri dish (B), and mounted on grips and submerged in saline during mechanical testing (C). Asterisk marks the agarose post around which the tissue ring contracted, and arrowhead marks the tissue ring. Scale bars: 2 mm (A); 4 mm (B); 4 mm (C).

Figure 2. Mechanical properties of CDM rings cultured in standard or supplemented growth medium

Tissue ring thickness (A), ultimate tensile strength; UTS (B), stiffness (maximum tangent modulus; MTM) (C), and failure strain (D), were measured after 8 days in culture. Tissue thickness is reported as mean $\pm$ S.E.M. and mechanical test parameter values are reported as mean $\pm$ S.D.; n=5 for the standard group, n=4 for the supplemented group. Asterisks indicate statistical differences between sample groups ($p < 0.05$).

Figure 3. Histological comparison of cell-derived tissue rings cultured in standard or supplemented growth medium

H&E micrographs of a cell-derived ring cultured in standard growth medium (A) or supplemented growth medium (B) for 8 days. Fast Green/Picrosirius Red micrographs of a cell-derived ring cultured in standard growth medium (C) or supplemented growth medium (D). Tissue samples are oriented with the center of the ring at the right in each image. Red staining indicates collagen. Scale bars = 50 μ m.

Figure 4. Mechanical properties of CDM, fibrin gel, and collagen gel rings

Tissue ring thickness (A), ultimate tensile strength; UTS (B), stiffness (maximum tangent modulus; MTM) (C) and failure strain (D) were measured after 7 days in culture. Tissue thickness is reported as mean $\pm$ S.E.M. and mechanical test parameter values are reported as mean $\pm$ S.D.; n=6 per group. Asterisks indicate a statistically significant difference between the denoted groups (p<0.05).

Figure 5. Stress-strain curves indicate failure properties of CDM, fibrin gel and collagen gel rings

Representative stress-strain data from a tissue ring sample in each experimental group (includes pre-cycles prior to pull-to-failure test).

Figure 6. Histological analysis of CDM, fibrin gel and collagen gel rings
H&E (A–C) and Fast Green/Picrosirius Red (D–F) micrographs of CDM (A, D), fibrin gel (B, E), and collagen gel (C, F) rings. Red staining in panels D–F indicates collagen. Tissue samples are oriented with the center of the ring at the right in each image. Scale bars = 50µm.

Figure 7. Histological analysis of cell distribution within fibrin and collagen gel rings 1, 2 and 3 days after cell seeding

H&E micrographs of fibrin gel (A–C) and collagen gel (D–F) rings cultured for 1 (A, D) 2 (B, E), and 3 (C, F) days. Tissue samples are oriented with the center of the ring at the bottom in each image. Scale bars = 100 μ m.

Figure 8. Matrix contributions to mechanical properties of CDM, fibrin gel, and collagen gel rings

Mechanical analysis of control tissue rings (black bars) and tissue rings treated with sterile deionized water to induce cell lysis (white bars) was performed to evaluate the biomechanical effects of the ECM, without active cell contraction. Tissue ring thickness (A), ultimate tensile strength; UTS (B), stiffness (maximum tangent modulus; MTM) (C) and failure strain (D) were measured after 7 days in culture. Tissue thickness is reported as mean $\pm$ S.E.M. and mechanical test parameter values are reported as mean $\pm$ S.D.; $n=4$ per group. Asterisks indicate a statistically significant difference between untreated and lysed samples ($p < 0.05$).