Benchmarks

Linear measurement of cell contraction in a capillary collagen gel system

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Three-dimensional collagen gel contraction is the standard assay utilized for functionally quantifying a variety of cell types, in particular smooth muscle cells (SMCs) and myofibroblasts. Here, we have developed a method to effectively reduce the three-dimensional parameters of the standard collagen gel into a single, linear measurement. Cell/collagen suspensions that are cast into glass capillary tubes provide several advantages over the well plate format, such as eliminating the need for digital imaging equipment and software to quantify the amount of cellular contraction. In addition, capillary tube gels require significantly fewer cells and far less reagents than standard methods.

Collagen gel contraction assays are widely used to determine contractile function in multiple cell types (1–3), including smooth muscle (4). Cells are suspended into a three-dimensional matrix containing polymerized type I collagen, cast into a well plate to form a disc, and diameter or surface area change in the gel is recorded over time. Macromolecular agents may be added to assess their effects on cellular contraction (3, 5–8). Measurement of a three-dimensional gel in one- or two-dimensional units introduces inaccuracy since volumetric changes are not considered. Distortion of the disc in three dimensions can also lead to inaccurate measurements, even though oil may be used to reduce friction and improve symmetry (9). We developed a simplified method to more accurately quantitate contractility by reducing the collagen gel dimensions to a single, linear measurement. This reduction was achieved by casting cell/collagen suspensions into commercially available glass capillary tubes, and measuring changes in length over time.

Primary human bladder–derived smooth muscle cells (SMCs) were suspended (500,000 cells/mL) in a rat tail collagen I solution (2.2 mg/mL; BD Biosciences, San Jose, CA, USA). MEM (Invitrogen, Carlsbad, CA, USA) supplemented with 1.8 mg/mL $NaHCO_3$ (Sigma-Aldrich, St. Louis, MO, USA) and 2.3 mg/mL L-glutamine (Invitrogen) was used as a diluent and pH was adjusted with 3.7 mg/mL HEPES (Invitrogen). Negative control hydrogels were supplemented with 5 μM EDTA (Invitrogen) to inhibit Ca^{2+}-dependent cellular contraction. For each replicate, 250 μL cell suspension was dispensed into a single well of a 48-well plate. From the same suspension, 30-μL aliquots were aspirated into 50-μL glass capillary tubes (outer diameter 1.372 mm, inner diameter 0.9 mm; VWR, West Chester, PA, USA). Plates and tubes containing cell/collagen suspensions were incubated at 37°C for 30 min to facilitate collagen polymerization. Next, the collagen gels were loosened from the well plate using a pipet tip, or loosened in the capillary tubes by blocking one end and gently applying air pressure through the other end with a small latex bulb (VWR) to reduce adhesion that can impede contraction. Serum-free DMEM (250 μL) was added to each well and incubated at 37°C in a humidified, 5% CO_2–containing atmosphere. Capillary tubes containing the loosened hydrogels were placed into a humidity chamber (150-mm cell culture dish with a container of sterile, deionized water) to prevent dehydration and incubated as described in the previous sentence. All gels were imaged using a Molecular Imager ChemiDoc XRS System (Bio-Rad, Hercules, CA, USA) at 0-, 24-, and 48-h times. Images were measured with ImageJ software version 1.40g and expressed in pixel units.

Ca^{2+}-dependent contraction occurred in both formats (Figure 1, A and B) and

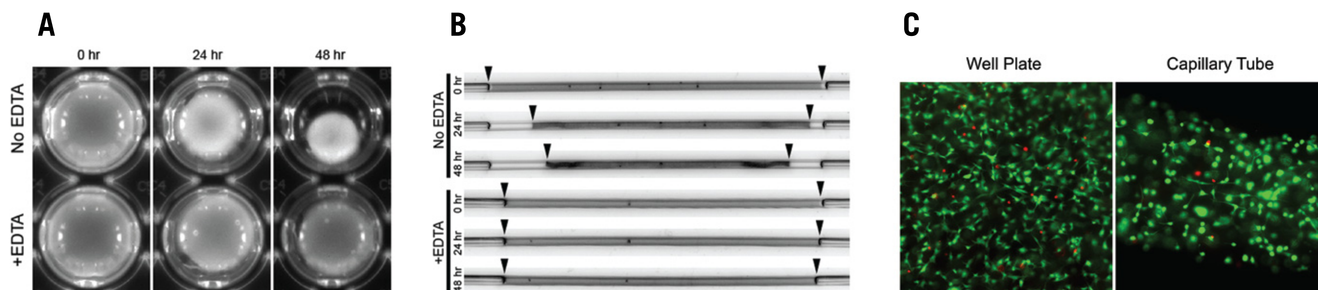

Figure 1. Directional contraction of collagen gels is determined by format dimensions. Human bladder SMCs were suspended at 500,000 cells/mL into a solution containing a final collagen type I concentration of 2.2 mg/mL, and cast into a 48-well plate (A) or 50-μL capillary tubes (B). Images were taken at 0-, 24-, and 48-h time points. In the well plate format, collagen gel surface area decreases over time (A), while decreases in length are measured in the capillary tube format (B). Arrowheads highlight the ends of the capillary gel (B). After the final 48-h time point measurement was taken, collagen gels containing SMCs were incubated in Calcein AM (green) and ethidium homodimer 1 (red) to assess live and dead cells, respectively (C).

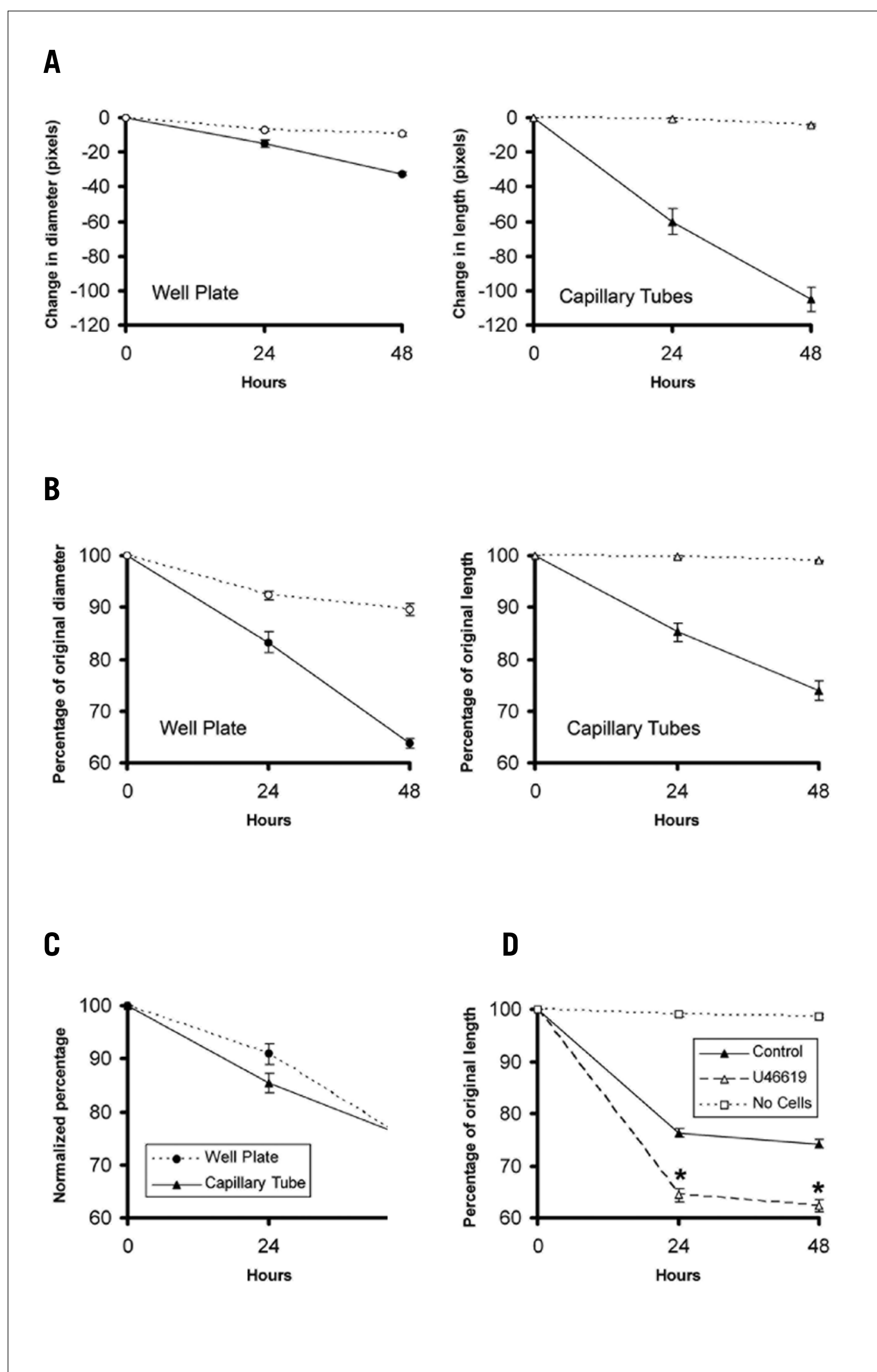

Figure 2. Collagen gel contraction is comparable between well plate and capillary tube formats. Average numerical changes in calculated gel diameter (well plates; $n = 3$) or length (capillary tubes; $n = 6$) over 24 and 48 h were normalized to the starting gel dimensions (all measurements in pixels, A). Solid lines represent the contracting gels, while negative controls (+EDTA) are represented by the dotted lines. Absolute numeric values (A) were converted to percentages of the original measurements at $t = 0$ (B). Percentage of contraction magnitude was compared between the well plate format (B, left panel) and capillary tubes (B, right panel), along with their respective +EDTA negative controls (dotted line). To account for the differences in Ca^{+2}-independent contraction between the two gel formats, percentages were normalized to the respective +EDTA negative controls (C), demonstrating that there are no significant differences in contraction percentage between the well plate and capillary formats ($P > 0.05$ at all time points). The average contraction magnitude in the capillary format was greater in gels containing 5 μM U46619 (dashed line; $n = 7$) compared with control gels (solid line; $n = 7$) at 24- and 48-h time points. Acellular collagen gels (dotted line; $n = 7$) did not contract (D). Asterisks (*) denote $P < 0.001$ compared with control gels (D). Error bars in all graphs represent SEM.

increased over time compared with the negative control (+EDTA). To assess cell survival, SMC-containing hydrogels were incubated 20 min in DPBS (Invitrogen) containing 1 μM calcein AM and 2 μM ethidium homodimer 1, provided in the Live/Dead Viability/Cytotoxicity Kit (Invitrogen). Two-channel fluorescent images were obtained using a Leica DMI4000B inverted microscope (Bannockburn, IL, USA) and merged using Simple PCI 6 software (Hamamatsu Corporation; Sewickley, PA). High cell viability and low cytotoxicity were observed in both formats (Figure 1C), indicating that capillary gels maintain cellular integrity without media addition.

Since contraction in the well plate occurred in three dimensions, we measured gel surface area as the closest approximation to volumetric units. Contraction in the capillary gel was restricted to a measurable linear (length) format with immeasurable changes in radius. Average numerical changes in calculated gel diameter (well plates; $n = 3$) or length (capillary tubes; $n = 6$) were normalized to each starting gel dimension at $t = 0$. Capillary gels display a much greater dynamic range than well plate gels (Figure 2A), suggesting the tube format is more accurate at detecting slight changes in linear gel dimensions.

Surface area and length measurements cannot be compared directly due to fundamental differences in unit dimensions. To make a direct comparison between the well plate and capillary tube formats, we elected to reduce the plate measurements from two dimensions to one dimension by calculating the diameter from the gel surface area. We chose this value to account for gel distortion in the well and to eliminate errors in approximating the center point of the gel. The absolute numerical changes of diameter or length of the gels were then converted to percentages of original measurements at $t = 0$ for comparison (Figure 2B). Comparing relative changes in gel dimensions, minimal differences in contraction magnitude between the two formats were revealed. To account for differences in Ca^{+2}-independent contraction between the two formats, percentages were normalized to the respective +EDTA negative controls (Figure 2C). Together, these data illustrate that three-dimensional (well plate format) and one-dimensional (capillary tube) contraction assay results are comparable.

Lastly, SMCs were suspended into collagen gels with or without the known

contraction agonist U46619. This synthetic analog of prostaglandin H_2 has been shown to mimic the biological action of thromboxane A_2 (10), acting as a potent stimulant of contraction in SMCs and smooth muscle tissues (7, 10–13). Capillary gels supplemented with 5 μM U46619 (Sigma-Aldrich) contracted to 64 ± 1.35% of the original length at 24 h, and 62 ± 1.24% of the original length by 48 h (Figure 2D). Control gels only contracted to 76 ± 0.09% and 74 ± 0.81% at the 24- and 48-h time points, respectively (Figure 2D). Unseeded collagen gels did not contract (Figure 2D).

In this study, we show that the standard collagen gel contraction assay can be adapted to a linear format without compromising the cellular dynamics of the system. While measurements taken in this study for both formats utilized digital imaging equipment and analytical software to compare the two methods, tube gels could be measured simply with a ruler or calipers. Accurate measurement of well plate gels with these tools is difficult. Additionally, capillary tube hydrogels utilize much smaller volumes, thus significantly reducing the amount of reagents required. This advantage is considerable when assaying expensive materials, or cells with limited availability. Finally, the capillary format lends itself to applying direct linear currents across the entire cell/collagen suspension to stimulate a contractile response.

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Competing interests

The authors declare no competing interests.

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