

Short communication

A multi-station culture force monitor system to study cellular contractility

Brian H. Campbell^a, William W. Clark^b, James H-C. Wang^{a,*}

^a *Musculoskeletal Research Center, Departments of Orthopaedic Surgery and Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA*

^b *Department of Mechanical Engineering, University of Pittsburgh, Pittsburgh, PA, USA*

Accepted 20 September 2002

Abstract

Cellular contraction contributes to the formation of scar tissue, which is characterized by an over-produced, disorganized collagen matrix. To study the contractility of cells *in vitro* and its potential contribution to scar tissue formation, we have developed a multi-station culture force monitor (CFM) system. This system consists of four vertical cantilever beams with semiconductor strain gages and a computerized data acquisition unit to monitor contractile forces of the cells in a collagen gel. Calibration showed that this system has a highly linear voltage–force relationship ($R^2 > 0.99$). Further, to demonstrate the applicability of this system, contractile forces of human skin fibroblasts in a collagen gel were measured. These fibroblasts were found to produce an average force of 0.2 nN/cell, which is consistent with the data in literature. The significant advantage of this CFM system is its ability to test multiple samples simultaneously. Therefore, the system can facilitate statistical design and analysis of experiments to study the effects of growth factors (e.g., TGF- β s) on cellular contraction and their potential role in scar tissue formation.

© 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Wound healing; Fibroblasts; Contraction; Culture force monitor

1. Introduction

Following injury, tissues often heal with formation of scar tissue (Adzick and Lorenz, 1994; Nedelec et al., 2000), characterized by an overproduced, disorganized collagen matrix. Cellular contraction, defined as the force cells exhibit on the extracellular matrix, plays a significant role in tissue wound healing (Nedelec et al., 2000). Studies suggest that high cellular contraction can lead to scar formation, whereas low contraction may impede wound healing and closure (Coleman et al., 1998; Nedelec et al., 2000). Since scar formation can be detrimental to tissue function, it is important to better understand the role of cellular contractility in tissue wound healing.

Two models used to study cellular forces are thin silicone membranes (Harris et al., 1980) and fibroblast populated collagen gels (FPCGs) (Bell et al., 1979; Murata et al., 1997). In the former model, cells are

plated on a thin silicone membrane. The formation of wrinkles under the cells demonstrates the ability of the cells to transmit forces to the membranes, which is a result of cellular motility (Harris et al., 1980). In the FPCG model, however, cellular contraction is semi-quantitatively determined by measuring the change in gel area over time, such that a smaller gel area corresponds to a greater degree of cellular contraction. Continued interest in cellular contraction and its role in wound healing led to the development of force monitor systems capable of quantifying cellular contraction of a single FPCG (Delvoye et al., 1991; Kolodney and Wysolmerski, 1992; Eastwood et al., 1994, 1996; Freyman et al., 2001).

In light of the need to study and statistically compare the effects of biological factors (e.g., growth factors) on cellular contraction, we have developed a multi-station culture force monitor (CFM) system. This paper presents the design and development of this new multi-station CFM system. Further, a sample measurement of fibroblast contraction is presented to demonstrate the feasibility of this system. This multi-station CFM system will be a useful tool to study the contractility of various

*Corresponding author. Tel.: +1-412-648-9102; fax: +1-412-648-2001.

E-mail address: wanghc@pitt.edu (J.H.-C. Wang).

types of cells, such as fibroblasts and endothelial cells, and the effect of growth factors (e.g., TGF- β) on cellular contraction.

2. Materials and methods

The new multi-station CFM system consists of the CFM apparatus (Fig. 1A) and a computerized data acquisition (DAQ) unit, capable of simultaneously monitoring contractile forces of multiple cell-populated collagen gels (CPCGs). The base of the CFM apparatus is an aluminum platform that can accommodate up to six CFM stations. The support rod assembly mounted to the base of the CFM provides a framework for positioning and fixing the beam-clamps (Fig. 1B). Further, tension units opposing the support rod assembly allow for fixation of one end on the gel, and provide a means for setting gel tension. The beam clamps, made of stainless steel, rigidly fix the beams and can move along the support rod assembly for proper positioning during experiments.

The beams, which are the sensors used to detect cellular contractile forces, are made of aluminum ($95.2 \times 5.0 \times 0.25 \text{ mm}^3$; Young's modulus, $E = 70 \text{ GPa}$), permitting sufficient beam strain under the expected cellular contractile forces in our system (~ 200 dynes). A small suture loop (braided polyester suture, 4-0; Ethicon, NJ) is tied to the beam through a 0.4 mm hole located 76.2 mm from the clamp. To measure beam strain, four semiconductor strain gages (gage factor = 140; resistance $\sim 400 \Omega$; Micron Instruments, CA), two on each side, in a full Wheatstone bridge configuration, were mounted on the beams located 1.6 mm from the clamp. In addition, a power supply (Hewlett Packard 6237B Power Supply) provides 4 V DC excitation voltage to all the semiconductor strain gages.

The silicone dishes ($70 \times 30 \times 10 \text{ mm}^3$), which contain the CPCGs, are custom made using a molding process as described previously (Wang and Grood, 2000).

Briefly, two silicone components (RTV ME 601A and 601B; Wacker Silicones Corporation, MI) are mixed in a ratio of 10:1. After being degassed, the silicone mixture is poured into a multiple-dish mold made of acrylic (Plexiglass). The silicone dishes obtained from this molding process are hydrophobic, thereby minimizing the attachment of cells and the collagen gel to the dish.

The force generated by the cells is transmitted from the CPCG to the beam via a piece of suture that connects the beam to the gel attachment unit (Fig. 1B). The gel attachment unit is composed of two parts, a porous vyon bar and a wire frame. The CPCGs polymerize into the porous vyon bars ($23 \times 8 \times 3 \text{ mm}^3$; Porvair Inc., NC) located at the ends of the silicone dish. The wire frames (0.36 mm stainless steel wire) extend vertically from the vyon bars to allow easy attachment to the beam and tension unit. As one end of the gel is fixed by the tension unit, the contraction of the CPCG is transmitted to the beam by the gel attachment unit and suture, causing the beam to deflect, which is monitored by the semiconductor strain gages (Fig. 1B).

The DAQ unit that receives the signal voltage is composed of a data acquisition (DAQ) card (6024E; National Instruments, TX), shielded connector block and cable (National Instruments, TX), and a personal computer. A Labview 5.1 program was developed to display and record the output voltage signals from the semiconductor strain gages. The Labview program can record signals at various time points and sampling rates. For experiments in this study, the voltage signals were recorded for 30 s at 10 Hz, every 5 min. Each data point corresponds to the mean of the respective 300 samples.

Calibration was performed for each individual circuit, i.e., the components required to monitor each individual beam deflection (beam, strain gages, wire, connecting block, computer, and Labview program). For calibration, the beams were set in a horizontal position, such that known weights could be suspended from the beams via the suture loops. The force from the weight causes beam bending, and the resulting beam strain is detected by the strain gages. Force is related to strain as shown

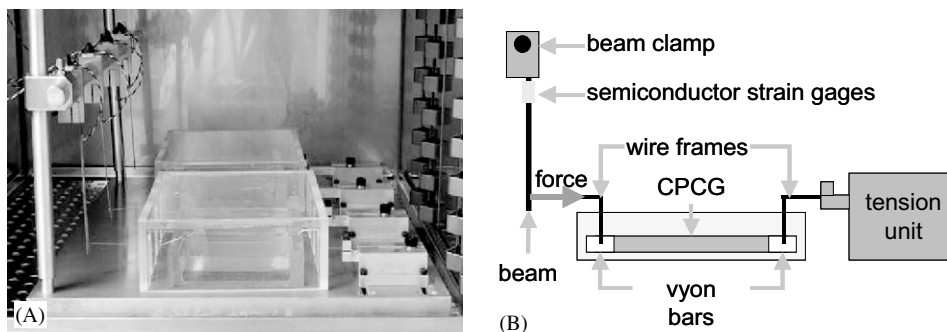


Fig. 1. Multi-station CFM apparatus is shown in an incubator during an experiment for measuring cellular contractile forces (A). The components of the CFM apparatus are illustrated (B). As shown, the cell-mediated contraction of the cell-populated collagen gel causes the beam deflection, such that the beam strain is detected by the semiconductor strain gages.

by the following equation, $P = Ebh^2\varepsilon/(6L)$, where P is a point force, E is Young's modulus, b is the beam width, h is the beam thickness, ε is the beam strain and L is the distance from the location of the point force to the location of the strain gages. Wires of different lengths were cut and weighed using an analytical balance (accuracy of 1.6 dynes; A-200D, Denver Instrument Company, CO). The weights of six wire pieces were 20, 39, 58, 77, 120, and 243 dynes, which covered the expected range of cellular contractile forces produced by about two million cells. For each applied weight, 300 voltage outputs were recorded and averaged to obtain one data point. This calibration was repeated three times. Linear regression yielded the voltage–force relationship for each circuit. Therefore, the calibration factor for each circuit, which is the slope from the linear regression of voltage and force data, could be used to calculate the force for a given voltage. Note that the calibration was performed in the same environment as that for monitoring cellular contractile forces, i.e., in an incubator at 37°C, 5% CO₂, and 100% humidity.

To show the applicability of the CFM system, human skin fibroblasts (HSFs) were used. The cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S; Invitrogen, CA) in 5% CO₂ at 37°C. The cells were sub-cultured to obtain sufficient numbers of cells for each experiment. A collagen gel solution was made by mixing collagen stock solution (~98% bovine collagen type I; Cohesion Technologies Inc., CA), 0.1 M NaOH, and 10X PBS in a ratio of 8:1:1. FPCGs were prepared in the rectangular silicone dishes by mixing 5 ml of the collagen gel solution (2.56 mg/ml) and 2×10^6 HSFs in 2 ml of medium. The FPCG solution was pipetted into the silicone dish and around the porous vyon bars. The FPCGs were incubated for 10 min to initiate gel polymerization, followed by addition of 7 ml of medium to each dish to allow FPCG to float in the medium. For all experiments in this study, DMEM was supplemented with 10% FBS and 1% P/S. The FPCGs were attached to the multi-station CFM system and a pretension of 2–10 dynes was set for each FPCG to ensure that the suture connections were taught. The FPCG contractile forces were monitored for up to 24 h. Voltage readings taken every 5 min at 10 Hz for 30 s were averaged to yield one data point, resulting in 12 data points/h.

3. Results

A CFM system with four circuits was successfully developed (Fig. 1A). The weight calibration of the system was proven to be effective and repeatable. With this method of calibration, it was shown that there was a highly linear voltage–force relationship for all four circuits ($R^2 > 0.99$) (Fig. 2). The linear relationship

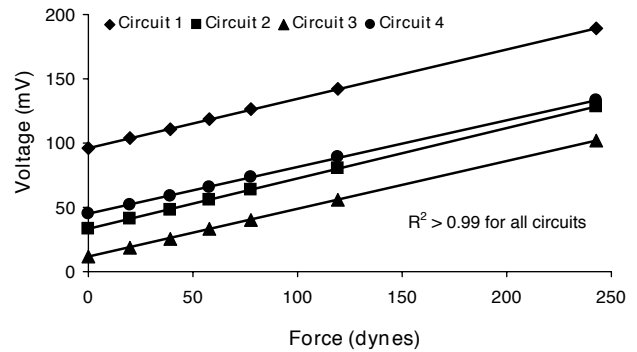


Fig. 2. Highly linear voltage–force relationship of each circuit of the CFM system can be used to directly calculate the contraction force from the change in voltage. To calibrate the CFM system, known weights (0–240 dynes) were added to the beams, and the voltage outputs were recorded. Note that the four lines were nearly parallel to each other, but their y-intercepts were different because the Wheatstone bridge for each circuit was not balanced.

means that the voltage output from the semiconductor strain gages is proportional to the force bending the beam throughout the range of the forces tested (≤ 240 dynes). Thus, the calibration factors, i.e., the slopes of the voltage–force lines from linear regression, for the four circuits, were found to be 2.63, 2.56, 2.70, and 2.78 dynes/mV.

In this CFM system, the fibroblasts visually deformed the gel into a parabolic shape, indicating they were contractile. Further, during the first 4 h, the cells produced forces that increased with time, then reaching a maximum equilibrium force of about 35 dynes after about 10 h (Fig. 3). The patterns of the force curves from the two FPCGs were similar, due to the same experimental conditions applied. Also, since the maximum contractile force of 35 dynes was produced by approximately 2×10^6 cells/gel, this corresponded to about 0.2 nN/cell.

4. Discussion

In this study, a multi-station CFM system was developed to measure contraction of multiple FPCGs simultaneously, expanding the capacities of existing force systems in measuring FPCG contraction (Delvoe et al., 1991; Kolodney and Wysolmerski, 1992; Eastwood et al., 1994; Eastwood et al., 1996; Freyman et al., 2001). This feature facilitates statistical design and analysis of experiments to study the effects of growth factors, such as TGF- β (Murata et al., 1997), on cellular contractility. In addition, calibration of the system demonstrated that there exists a highly linear relationship ($R^2 > 0.99$) between the voltage output and applied force, thus allowing for accurate calculation of contraction force from a given change in voltage.

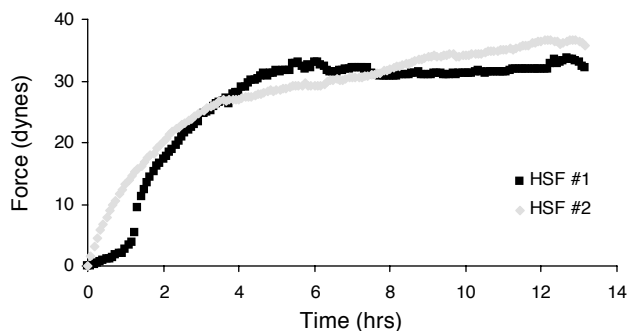


Fig. 3. CFM system successfully measured the contraction of 2 million HSFs in a collagen gel, demonstrating the applicability of this system to study cellular contraction. Most noteworthy, however, is that under the same experimental conditions, the two fibroblast-populated collagen gels (FPCGs) produce similar contraction curves.

To demonstrate the applicability of this system in measuring FPCG contraction, HSFs were used because they are readily available, easy to culture, and most importantly, their contraction is well documented (Delvoye et al., 1991; Kolodney and Wysolmerski, 1992; Eastwood et al., 1994; Eastwood et al., 1996; Freyman et al., 2001). Using this system, HSFs were found to produce 0.2 nN/cell, on average. The average contraction force found in this study is consistent with previous studies using similar devices, which showed that skin fibroblasts in a collagen gel produce between 0.1 and 10 nN/cell (Delvoye et al., 1991; Kolodney and Wysolmerski, 1992; Eastwood et al., 1994, 1996; Freyman et al., 2001). Further, the pattern of contraction, i.e. the overall shape of the contraction curve, is similar to that found in previous studies for HSFs (Eastwood et al., 1994, 1996; Freyman et al., 2001). Note, however, that similar to previous force systems, our CFM system only measures contraction of a population of cells in a collagen matrix, not of the individual cells. Therefore, the value of force/cell can only be considered as a rough estimate for comparison.

Similar to other sensitive devices, the major constraint of this system is the initial preparation and setup. Since the forces being measured are very small (< 50 dynes), any perturbations (e.g., environmental noise) in the system can be problematic. To minimize these effects, a standardized protocol has been established to use this system. Further, the gels are examined to ensure that they properly detach from the sides of the silicone dish, allowing for the FPCG contraction to be measured. As demonstrated by the similar contraction patterns of the two FPCGs (Fig. 3), the perturbations in this system can be minimized.

As cellular contraction is a key component in wound healing, this new multi-station CFM system will be a useful tool to study the effects of growth factors (e.g., TGF- β) on cellular contraction. In addition, it can be used to examine the relationship between cellular

contraction, cell proliferation, and collagen production, which is important in understanding the mechanisms for scar tissue formation.

Acknowledgements

BHC was supported by the Wellington C. Carl Scholarship from The Pittsburgh Foundation. This study was supported in part by the Whitaker Biomedical Engineering Grant, Arthritis Investigator Award, and NIH grant AR47372-01 (JHW). We thank Drs. Woo and Eastwood for their helpful discussion during the development of this CFM system. We also thank Philip Magcalas and Beth Kirkpatrick for their technical assistance in this study.

References

- Adzick, N.S., Lorenz, H.P., 1994. Cells, matrix, growth factors, and the surgeon. The biology of scarless fetal wound repair. *Annals of Surgery* 220 (1), 10–18.
- Bell, E., Ivarsson, B., Merrill, C., 1979. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proceedings of the National Academy of Sciences of the United States of America* 76 (3), 1274–1278.
- Coleman, C., Tuan, T.L., Buckley, S., Anderson, K.D., Warburton, D., 1998. Contractility, transforming growth factor-beta, and plasmin in fetal skin fibroblasts: role in scarless wound healing. *Pediatric Research* 43 (3), 403–409.
- Delvoye, P., Wiliquet, P., Leveque, J.L., Nussgens, B.V., Lapiere, C.M., 1991. Measurement of mechanical forces generated by skin fibroblasts embedded in a three-dimensional collagen gel. *Journal of Investigative Dermatology* 97 (5), 898–902.
- Eastwood, M., McGrouther, D.A., Brown, R.A., 1994. A culture force monitor for measurement of contraction forces generated in human dermal fibroblast cultures: evidence for cell-matrix mechanical signalling. *Biochimica et Biophysica Acta* 1201 (2), 186–192.
- Eastwood, M., Porter, R., Khan, U., McGrouther, G., Brown, R., 1996. Quantitative analysis of collagen gel contractile forces generated by dermal fibroblasts and the relationship to cell morphology. *Journal of Cellular Physiology* 166 (1), 33–42.
- Freyman, T.M., Yannas, I.V., Pek, Y.S., Yokoo, R., Gibson, L.J., 2001. Micromechanics of fibroblast contraction of a collagen-GAG matrix. *Experimental Cell Research* 269 (1), 140–153.
- Harris, A.K., Wild, P., Stopak, D., 1980. Silicone rubber substrata: a new wrinkle in the study of cell locomotion. *Science* 208 (4440), 177–179.
- Kolodney, M.S., Wysolmerski, R.B., 1992. Isometric contraction by fibroblasts and endothelial cells in tissue culture: a quantitative study. *Journal of Cell Biology* 117 (1), 73–82.
- Murata, H., Zhou, L., Ochoa, S., Hasan, A., Badiavas, E., Falanga, V., 1997. TGF-beta stimulates and regulates collagen synthesis through TGF-beta1-dependent and independent mechanisms. *Journal of Investigative Dermatology* 108(3), 258–262.
- Nedelec, B., Ghahary, A., Scott, P.G., Tredget, E.E., 2000. Control of wound contraction. Basic and clinical features. *Hand Clinics* 16 (2), 289–302.
- Wang, J.H., Grood, E.S., 2000. The strain magnitude and contact guidance determine orientation response of fibroblasts to cyclic substrate strains. *Connective Tissue Research* 41 (1), 29–36.