

Technical note

# A multi-station dynamic-culture force monitor system to study cell mechanobiology

Katherin A. Peperzak, Thomas W. Gilbert, James H.-C. Wang\*

*Mechanobiology Laboratory, Musculoskeletal Research Center, Departments of Orthopaedic Surgery, Bioengineering and Mechanical Engineering, University of Pittsburgh Medical Center, E1641 Biomedical Science Tower, 210 Lothrop Street, PO Box 71199, Pittsburgh, PA 15213, USA*

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## Abstract

To study mechanobiological responses of cells, a dynamic-culture force monitor (D-CFM) system has been developed. The D-CFM extends our previous work to measure contractile forces of a cell-populated collagen gel (CPCG) using a cantilever beam with semiconductor strain gauges. Linear actuators are used in the system and are computer controlled using a LabVIEW interface to independently apply precise motion waveforms to multiple CPCGs. The feasibility tests showed that the new system can detect the differences in force patterns resulting from different motion waveforms imparted to the CPCG. This new system will facilitate the study of the effects of dynamic mechanical loading on cells, remodeling of extracellular matrix, and cell–matrix interactions in vitro.

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## 1. Introduction

The mechanical environment to which cells are exposed greatly influences their behavior [1–4]. To increase the understanding of how mechanical loading affects cell behavior, a number of in vitro models have been developed [2,4–11]. These systems have varied greatly in their design and functionality, but can generally be fit into categories based on the substrate used to apply strain to the cells and the mechanism by which that strain is produced. Elastic membranes have been one of the most common substrates used. While this approach can provide valuable information about cell behavior, no information can be obtained about the interactions between the cells and the extracellular matrix that would make up their environment in vivo. To address cell–matrix interactions, the collagen gel model, which was first presented by Bell et al. [12], has been incorporated into a number of systems which are

generally characterized by the means of applying the strain and by whether they have the ability to measure the load in the matrix. With these systems, it has been shown that mechanical stretching of fibroblast-populated collagen gels (FPCGs) induces cell and collagen alignment along the stretching direction [2,5] and regulates protein synthesis [2,3,13]. A notable example of these in vitro models is the tensioning-culture force monitor (t-CFM) developed by Eastwood et al. [5]. This system can apply precise low frequency (on the order of 10 min to several hours for one cycle) loads to a CPCG using motors, and monitors the forces resulting from deformation of the CPCG, as well as forces from contraction by the cells within the matrix. A number of other systems have also been developed to apply cyclic loads to CPCGs with a more physiologically relevant frequency (on the order of 0.5 Hz) [6,7]. However, these devices are either not designed to record the load in the scaffold or do not have the sensitivity to measure cellular contractility. Therefore, the current work describes a new, multi-station dynamic-culture force monitor (D-CFM), which has the ability to independently apply precise motion waveforms to

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

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

each sample, while simultaneously measuring the forces of each CPCG due to the combination of deformation in the gel and the contractility of the cells.

## 2. Materials and methods

### 2.1. D-CFM system

The D-CFM is an extension of the culture force monitor (CFM) system developed in our research center [14]. The CFM apparatus is capable of monitoring contractile forces of multiple CPCGs simultaneously. The base of the apparatus is an aluminum platform that can accommodate up to four stations. Each CPCG is connected to an aluminum beam ( $95.2 \times 5.0 \times 0.25$  mm) on one side and to a rigid mount on the other with a stainless steel wire frame (0.36 mm stainless steel wire) which attaches to a porous vyon bar ( $23 \times 8 \times 3$  mm; Porvair Inc., NC). The porosity allows the collagen to flow into the vyon bars so that there is a more rigid fixation between the gel and the vyon bar once polymerization occurs. The CPCG was formed in a custom rectangular silicone dish with inside dimensions of  $70 \times 30 \times 10$  mm. The dishes were made by mixing two silicone components (RTV ME 601A and B; Wacker Silicone Corporation, MI) and pouring the mixture over an acrylic mold. Semi-conductor strain gages (gage factor = 140; resistance  $\sim 400$   $\Omega$ ; Micron Instruments, CA) were mounted onto the aluminum beams to determine strains resulting from forces that develop in the CPCG. Standard curves were created by hanging weights on each beam so that the current produced by the semi-conductor strain gage could be related to force. In previous experiments, the CFM measured reproducible forces for FPCGs for periods up to 14 h [14].

For the D-CFM, the rigid mounts for the stationary vyon bars were replaced with linear actuators (#43H4N-05, Haydon Switch and Instrument) to apply specified displacements to the CPCG (Fig. 1). The linear actuators are made of stable materials (e.g. thermoplastics and stainless steel), which are able to withstand the standard incubator conditions ( $37$  °C and a humidified atmosphere with 5%  $\text{CO}_2$ ). The linear actuators have a resolution of  $0.3$   $\mu\text{m}$  per step, and have position accuracy within  $20$   $\mu\text{m}$ . Power is supplied to the linear actuators using a micro-stepping power drive (National Instruments MID-7604). The motion control card (National Instruments PCI-7334), which receives signals from the computer and relays them to the specified linear actuator, is housed in a PCI expansion unit (Magma 2 slot CardBus-to-PCI Expansion System). Custom LabVIEW programs are used to define the motion waveform for each linear actuator. The programs provide the functionality to allow the

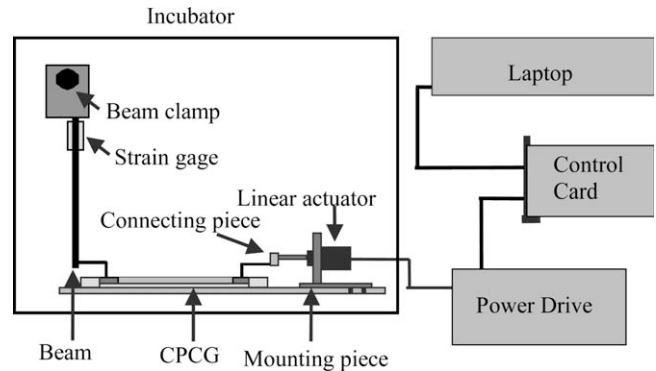


Fig. 1. The illustration of the D-CFM system. Using a custom-designed LabVIEW interface, the linear actuator imparts a defined motion to a cell-populated collagen gel (CPCG), and the force generated in the CPCG is monitored by the semi-conductor strain gages on the beam and recorded by a laptop computer.

user to set the gauge length of the specimen, and then apply either static or cyclic displacements to the CPCG. To apply a specific cyclic motion, the program prompts the user to define the amplitude (mm), period (seconds), and waveform (sine, triangle, or square). The displacement and resulting force of the CPCG as a function of time are saved to a text file for post-processing.

### 2.2. Feasibility experiments

To determine the feasibility of the D-CFM for CPCG experiments, the device was used to apply different shapes of waveforms (sine, triangle, and square) with magnitudes of 3 or 5 mm of displacement. The collagen gel solution (2.56 mg/ml) was first prepared by mixing the collagen stock solution ( $\sim 98\%$  bovine collagen type I; Cohesion Technologies Inc., CA), 0.1 M NaOH, and 10X PBS (Invitrogen, Carlsbad, CA) in a ratio of 8:1:1. The cells used in the experiment were primary human patellar tendon fibroblasts (HPTFs) obtained from trimmings of a bone–patellar tendon–bone graft for ACL reconstruction. The cell suspension was prepared by adding  $4 \times 10^6$  HPTFs to 6.6 ml of Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (DMEM+) (Invitrogen). To the cell suspension, 1.4 ml of the collagen gel solution was added and the mixture was pipetted over the porous vyon bars to fill the rectangular silicone dish (8 ml/dish). The pre-gel was then incubated for 10 min, after which 3 ml of DMEM+ was added to each dish to allow the gel to float. The collagen gels were attached to the D-CFM and a pre-tension of 10–20 dyn was applied manually.

The linear actuator was fully extended during the gel polymerization, which placed the vyon bar 12 mm from the edge of the silicone dish. After polymerization for 1 h, the gel was stretched repeatedly using first sine

and then triangle waveforms to determine if different loading patterns can be detected. For each waveform, an experiment was run at a period of 20 s with amplitudes of 3 and 5 mm to assess the working range of the system. Each combination of motion waveform, period, and amplitude was cycled from 3 to 30 min. The forces were monitored to determine the stability of the measurements and the integrity of the samples after loading. A minimum of 30 min of recovery time was allowed before each loading regime. Square waves were attempted, but the system did not work well at the high strain rate, with the gel becoming detached from the vyon bars or the vyon bars contacting the side walls of the dish.

### 3. Results

A dynamic-culture force monitor was successfully developed to apply precise displacement waveforms to multiple CPCGs, while simultaneously measuring the forces in the CPCGs. The feasibility experiments showed that the patterns of forces measured from CPCGs corresponded to the defined motion patterns, as expected (Fig. 2). It is shown that for cycling of 3 mm amplitude, the sine wave of the imparted motion directly correlated to the force pattern (Fig. 2a). A similar motion-force relationship can be seen for the triangle wave (Fig. 2b). The pattern correlation was present for all motion waveforms, amplitudes, and periods. Furthermore, it was found that with an amplitude of displacement of 5 mm, the maximum force increased by 2 dyn/h. This indicates that the system is capable of detecting forces resulting from external loading as well as from contraction of cells within the gels.

### 4. Discussion

A new multi-station D-CFM system has been presented, and the feasibility of applying this system to multiple CPCGs has been demonstrated. It is shown that the system can provide full motion control of the high speed linear actuators with several motion waveforms (e.g. sinusoidal and triangle waves), which can be conveniently defined by inputting the type of wave desired, displacement, and period. The D-CFM can accurately measure the forces of the CPCGs due to the applied motion as well as cell contraction. A significant advantage of the D-CFM is its ability to simultaneously monitor the forces of multiple CPCGs subjected to a wide range of complex but defined loading regimes (e.g. sinusoidal wave with various amplitudes and frequencies). It should be noted, however, that because of many factors involved, such as organization of collagen fibers within the gel and the cells' interac-

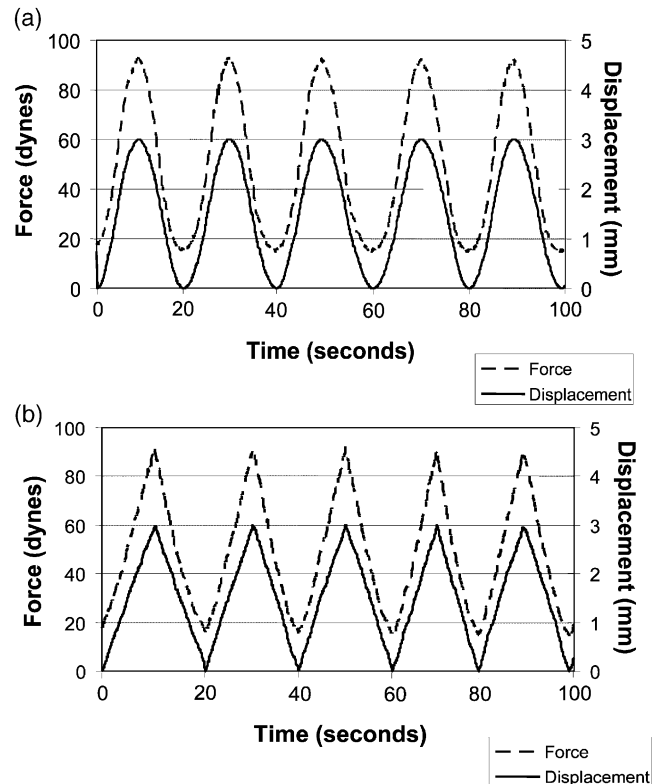


Fig. 2. The feasibility experiments of the D-CFM system. (a) A sine motion waveform was imparted to stretch the collagen gel. The resulting force of the CPCG was recorded as an output. (b) A triangle motion waveform was applied to the CPCG, and the resulting force of the CPCG was again recorded. It is shown that in both cases, the motion wave form corresponds to the force wave form, except that the force was shifted about 20 dyn, a pre-tension that was applied to the CPCG.

tions with the fibers, it is difficult to determine the magnitude of strain the cells are subjected to.

The D-CFM will facilitate our study of the effects of different loading regimes on biological responses of cells and matrix remodeling in vitro. First, with its ability to apply precise displacements to the CPCGs, it is possible to determine how different loading regimens affect cell contractility, an important factor in the formation of scar tissue [15]. Second, using the D-CFM system, the effect of mechanical loading on the remodeling of extracellular matrix can also be studied in a controlled manner. Many studies have shown that when fibroblasts are seeded in a collagen matrix and subjected to a load, the fibroblasts and collagen fibers both align parallel to the direction of the applied load [2,5]. Third, because the D-CFM can measure forces transmitted through the matrix (e.g. collagen gel), the mechanical behavior of the matrix can be characterized as remodeling occurs due to biological responses of cells to applied mechanical loading. Fourth, the D-CFM can be used to study the interactions between

cells and extracellular matrix. An example of the interaction is between integrins and their receptors, which are known to be involved in the reorganization of collagen fibers [16–18]. Finally, with this system, the combined effect of mechanical and biological factors can be investigated. For example, various growth factors (e.g. TGF- $\beta$ ) can be applied to determine if their effects depend on mechanical loading conditions [19–21].

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