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Technical note

Characterization of the Flexcell™ Uniflex™ cyclic strain culture system with U937 macrophage-like cells

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Abstract

Mechanical forces alter many cell functions in a variety of cell types. It has been recognized that stimulation of cells in culture may be more representative of some physiologic conditions. Although there are commercially available systems for the study of cells cultured in a mechanical environment, very little has been documented on the validation techniques for these devices. In this study, Flexcell's™ recently introduced Uniflex™ cyclic strain system was programmed to apply 10% longitudinal sinusoidal strain (0.25 Hz) for 48 h to U937 cells cultured on Uniflex™ plates. Image analysis was employed to characterize the actual strain field. For a chosen amplitude of 10% the applied strain was highly reproducible and relatively uniform ($10.6 \pm 0.2\%$) in a central rectangular region of the membrane (dimensions of $9.2 \pm 2 \times 13.6 \pm 0.8\,mm^2$). The strain increased the release of IL-6, esterase and acid phosphatase activity ($p < 0.05$) from adherent U937 cells. Cells also displayed altered morphology, aligning and lengthening with the direction of strain, whereas static cells maintained a round appearance showing no preferred orientation. These data indicate that cyclic mechanical strain applied by the Uniflex™ strain system modulates U937 cell function leading to selective increases in enzymatic activities as well as orientation in a favored direction.

Keywords: Macrophage; Mechanical test; Esterase; Uniaxial strain; Siloxane

1. Introduction

When trying to model in vivo cellular responses, it has become increasingly apparent that static cultures are less representative of physiologic conditions than cultures exposed to mechanical deformation [1]. Banes et al. introduced the first commercially available flexible-bottomed cell culture plates for mechanostimulation [2], providing a model system for investigating cell responses to specific biological settings. Currently, the Flexercell™ Strain Unit is the most widely used laboratory apparatus for the delivery of controlled in vitro mechanical strain to cultured cells (reviewed in [3,4]). In the first biaxial strain system developed by Flexcell™, adherent cells were cultured on Bioflex™ plates with flexible membranes that were deflected downward using negative pressure, thereby imparting strain to the culture layer. Williams et al. reported a theoretical analysis on the strain profiles of a circular elastic diaphragm [4] and characterization was further performed by Gilbert et al. who demonstrated that the strain gradient of the Bioflex™ well was equibiaxial at the center but approached pure uniaxial strain in the

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radial direction at the well edge [5]. Recently, Flexcell™ introduced a method to generate predominantly uniaxial strain by selectively controlling the portion of the membrane that is exposed to a regulated vacuum. When a pressure differential is applied to a Uniflex™ plate in this system, the cell culture membrane deforms across the face of an ArcTangle™ post insert, creating a region of strain on the membrane that is primarily uniaxial.

Techniques for the application of longitudinal strain include substrate end displacement and substrate flexure. Previous loading systems used a complex motorized piston/linkage arrangement [6] or the mechanically simpler spring-loaded pulley/cable arrangement [7]. However, these systems saw undesired end effects and heterogeneity in the strain fields. Although they may have proven useful for the collection of biological data, it is difficult to interpret results primarily because the accuracy and reproducibility of strain profiles have not been clearly defined [8,9]. Matsumoto et al. used a custom-built longitudinal cyclic stretch cell culture system to illustrate the extreme sensitivity of U937 cells to strain, which resulted in inhibition of differentiation and retardation of phagocytosis [10]. Other groups have used stepper-motor technology to impose precisely defined strain on cell culture substrates [11,12]. These computer-controlled devices may have demonstrated success; however, their limited availability and complicated procedures have restricted their general use.

The objective of this study was to accurately characterize the strain field in a Uniflex™ culture plate, as this has not yet been reported using the Flexcell™ Uniflex™ cyclic strain culture system. The functional and morphological responses of the macrophage-like U937 cell system, cultured on uncoated siloxane, to a well-characterized strain field were analyzed. Cell attachment, protein levels and markers of cell activation were measured in response to strain to demonstrate that U937 cells clearly respond to longitudinal stretch.

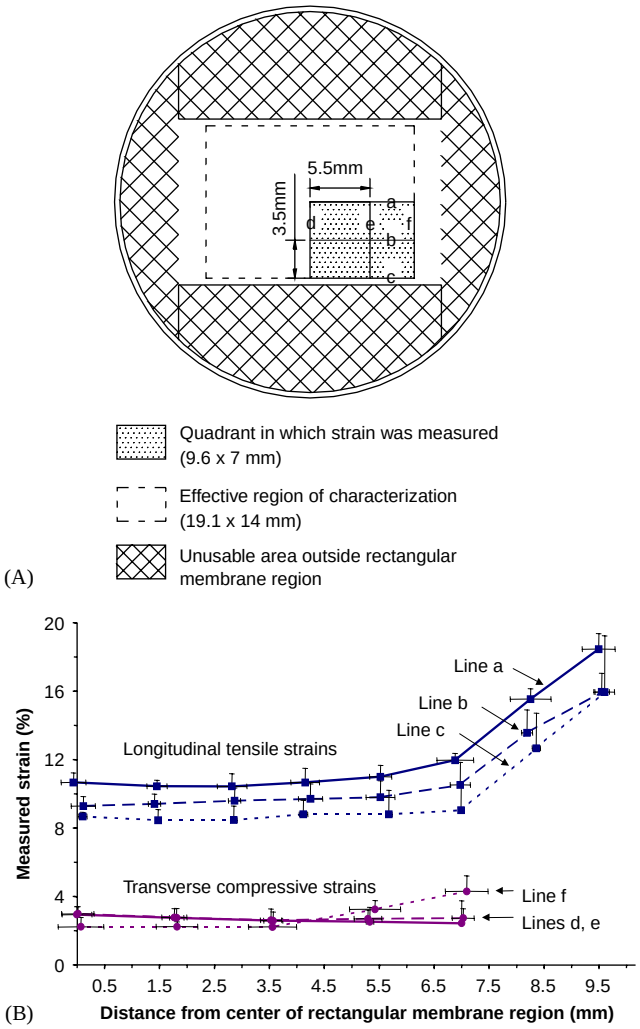

Fig. 1. (A) Schematic of the Uniflex™ well showing the rectangular membrane region on which cells are attached and strained. Linear orthogonal strains were measured in one quadrant of this region as described in detail in materials and methods. The symmetry about the midlines of the rectangular region was verified, and therefore strain magnitudes in any other quadrant of the 'effective region of characterization' can be obtained by translation to those in the measured quadrant. Lines (a), (b) and (c) indicate that line of measured points for longitudinal plots in Fig. 1B. Lines (d), (e) and (f) indicated the line of measured points for the transverse plots in Fig. 1B. (B) Graph showing the measured linear strains along the lines depicted in (A) for a setting of 10% strain on the Flexcell™ Strain Unit.

2. Materials and methods

Unless otherwise specified, all reagents were purchased from Sigma Chemical Company, St. Louis, MO. Uniflex™ culture plates were used with ArcTangle™ Loading Stations™ on a standard BioFlex™ baseplate within a Flexercell™ FX-4000 Tension Plus Strain Unit (all of Flexcell™ Int. Corp., McKeesport, PA).

2.1. Stretch device strain validation

Cells were grown in the usable region of the Uniflex™ membranes (non-hatched region in Fig. 1A). The membrane is placed over a specially shaped insert such that the end regions to the left and right of this rectangle are deflected downward when negative pressure is applied. To characterize the strain in the centrally located rectangular region of the Uniflex™ well, rows of 0.4 mm dots (68 in total) were drawn on one quadrant with ink ($3.5 \times 5.5\,mm^2$ region indicated in Fig. 1A) and imaged with a CCD camera at static Flexcell™ strains of 0% and 10%, as entered into the Strain Unit. The center of each of the dots drawn on the membrane was located by a pixel intensity-weighted center-of-mass algorithm with and without strain. Linear strains were calculated as the average percent difference between the displacement of a given dot and the displacements of its two neighbors along various lines, longitudinal and traverse to the long axis of the rectangular region, on three different membranes. This was repeated 3–5