NUTRIENT MEDIUM REACTIVE STRESSES IN A CELL CULTURE MECHANOSTIMULUS SYSTEM DELIVERING HOMOGENEOUS EQUIBIAXIAL STRAINS

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INTRODUCTION: Several novel cell culture mechanostimulus designs [1,2,3] have recently been introduced to obviate the strain field inhomogeneity and anisotropy seen with earlier designs [4,5] reliant on transmural pressure differentials applied across a diaphragmatic culture substrate. Despite the apparent improvement in substrate kinematic uniformity, these new designs introduce heretofore unencountered patterns of fluid motion in the overlying liquid medium, thereby developing novel patterns of reactive normal and shear stresses at the culture surface. We here report how duty cycle parameters influence the reactive fluid stresses in one increasingly popular such design, involving a membrane-like circular substrate which is radially stretched as it is distended by pulsatile motions of a lubricated, flat circular platen.

METHOD: The apparatus considered is that originally introduced by Schaffer et al. [2], in which axisymmetric substrate distentions are achieved by cam-driven vertical excursions of a flat-ended, rounded-lipped cylindrical platen contacting the substrate undersurface (Fig 1). Nutrient medium flow fields were determined by finite element solution of the Navier-Stokes equations [6], the nutrient medium being modeled as a Newtonian fluid of density ρ and viscosity μ. Ranges of variation for the system operational parameters were: 0.180 to 3.97 mm for A (corresponding to peak substrate strains from 0.29 to 6.43%), 0.25 to 4 Hz for f, and 3 to 9 mm for the resting depth h of the nutrient. Each of these three parameters was individually perturbed in two increments, both upward and downward, from baseline (A,f,h) = (1.11, 1, 6).

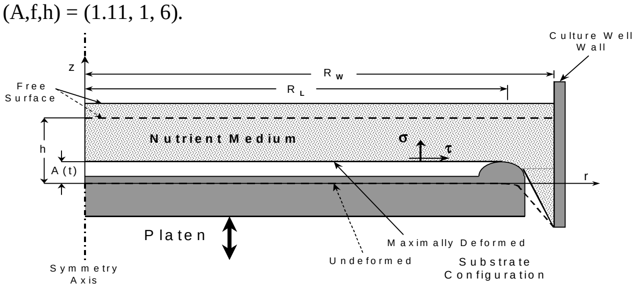

Figure 1. Schematic diagram (axisymmetric geometry) of the mechanostimulus system. Physically, R_W = 38 mm, R_L = 31.8 mm, $\rho = 1.03\times10^3$ kg/m^3, μ = 0.02 poise.

RESULTS: Reactive shear stresses were developed at the culture surface due primarily to the radial gradient of the radial component of fluid velocity (arrows, Figure 2) superimposed on the bolus-like vertical pulsations of the fluid mass. Compared to the low values prevailing over most of the substrate through most of the duty cycle, shear stress was almost discontinuously severe near the platen lip (Fig 3). By contrast, reactive normal stresses remained nearly uniform across the culture surface, showing only subtle temporal fluctuations due to the excursions of immersion depth (near-hydrostatic pressure distribution, shaded isobars in Fig 2) accompanying fluid egress/ingress from the "crevice" region lying just outward from the platen lip. For the respective parametric ranges considered, both the instantaneous local maxima and the temporal-spatial averages of unsteady reactive fluid stress departures from baseline depended most sensitively (6.5-fold variation) on frequency, intermediately (3.7-fold) on amplitude, and least sensitively (1.4-fold) on nutrient depth (Table1).

DISCUSSION: As with contemporary systems delivering more heterogeneous strain inputs [6], the prevailing magnitudes for shear stress in the present system were several orders of magnitude lower than for normal stress. However, given the exquisite shear sensitivity of various mechanoreceptors and transduction pathways, even shear magnitudes on the order of tenths of a dyne/cm^2 merit avoidance where possible. To that end, it is clearly preferable in the present system to avoid culture proliferation onto the peripheral region of the substrate, and to operate the system at low frequency. (By implication, usage of abrupt excitation transients such as square or sawtooth waves would be particuarly deleterious.) Given those modest precautions, however, the present data suggest that reactive fluid stress is not likely to be a major source of mechanostimulus artifact for this particular apparatus design.

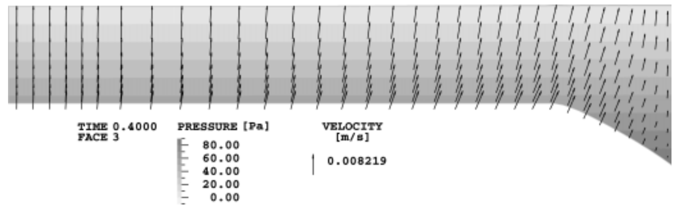

Figure 2. A typical nutrient medium instantaneous flow field (velocity shown vectorially, pressure shown shaded) late in the upward phase of platen excursion.

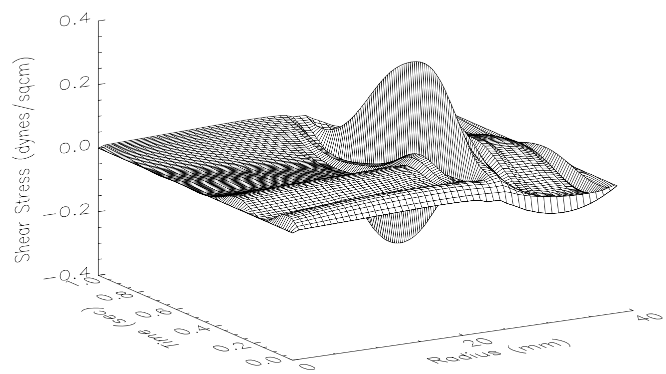

Figure 3. Temporal/spatial variation of reactive fluid shear stress throughout a typical (periodic) duty cycle for the baseline case (A=1.11 mm, f=1 Hz, h=6 mm). The large rise in shear stress occurs near the edge of platen contact.

Case	A	f	h	ε_{MAX}	σ_{MAX}	τ_{MAX}	σ_{AVG}	τ_{AVG}
Base	1.11	1	6	1.8	93.57	0.334	11.86	0.035
A↑	2.65	1	6	4.3	224.7	0.804	27.86	0.087
A↑↑	3.97	1	6	6.4	323.4	1.242	39.44	0.151
A↓	0.64	1	6	1.0	53.79	0.192	6.871	0.020
A↓↓	0.18	1	6	0.3	15.10	0.054	1.944	0.005
f↑	1.11	2	6	1.8	95.10	0.278	15.46	0.097
f↑↑	1.11	4	6	1.8	76.81	2.175	25.19	0.602
f↓	1.11	½	6	1.8	93.67	0.323	11.04	0.021
f↓↓	1.11	¼	6	1.8	93.71	0.322	10.84	0.015
h↑	1.11	1	7.5	1.8	92.78	0.317	11.93	0.042
h↑↑	1.11	1	9	1.8	92.04	0.302	12.03	0.049
h↓	1.11	1	4.5	1.8	94.58	0.352	11.85	0.030
h↓↓	1.11	1	3	1.8	96.29	0.464	11.97	0.107

Table 1. Peak substrate strain ε_{MAX} (%), and peak ($_{MAX}$) and average ($_{AVG}$) unsteady reactive fluid normal (σ) and shear (τ) stresses (dynes/cm^2).

REFERENCES: [1] Williams et al., *J. Biomech. Engr. 114*:377, 1992; [2] Schaffer et al., *JOR 1* 2709, 1994; [3] Stage Flexer, Flexcell Int'l (unpublished) 1997; [4] Banes et al., *J. Cell Sci. 75*: 35, 1985; [5] Winston et al., *J. Appl. Physiol. 67*:397, 1989; [6] Brown et al., *Am.J.Med.Sci.* (in press).
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