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Short communication

Oscillating Couette flow for in vitro cell loading

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Abstract

Synovial joints are loaded by weight bearing, stretching, and fluid-driven shear. To simulate in vitro fluid-driven shear, we developed an "oscillating Couette flow mechanical shear loader". Oscillating Couette flow mimics relative motion of articular surfaces; hence, characterizing flow-induced shear by the loader enhances understanding of mechanotransduction in the joint tissue. Here, the analytical and computational models for an oscillating Couette flow were used to predict time-varying shear distribution on a plate surface, applying numerical simulation to evaluate the effects of finite plate dimension in a 2D flow. Shear stress on the plate was significantly different from that in simpler models (unbounded plates and viscous low-frequency flow). High-stress spots appeared near the leading and trailing edges of a moving plate, and a relatively uniform shear region was restricted to the interior area. Stress prediction in an example experimental geometry is presented, where the frequency and finite width effects are feasibly accounted.

Keywords: Oscillating flow; Shear stress; Computational fluid dynamics; Synovial joint; Mechanotransduction; Articular surface

Biological tissues experience mechanical loads induced by pressure, stretching, or fluid circulation unique to each tissue. Synovial joints are covered by articular cartilage, a stratum of specialized hyaline cartilage of varying thickness, and sliding contact under load is facilitated by the viscous synovial fluid that acts as a lubricant and shear transducer. A variety of geometric figures are referred to classify synovial joints including cylindrical, conical, ellipsoid, and ovoid. One of the simplest representations uses finite planes to approximate congruent articular surfaces. The area of contact is strictly confined between cartilaginous planar surfaces, and the normal motion of synovial fluid can be viewed as oscillating Couette flow in a finite domain. An intra-articular structure such as an articular disc and meniscus limits the extent of the region with a very low coefficient of friction, and relatively higher stress spots are anticipated at the edge of the articulating surface.

To understand the loading effects, cells isolated from normal or diseased tissue are often grown under mechanical stimuli. An ideal in vitro cell loader for synoviocytes or chondrocytes should induce fluid-driven shear in a physiologically relevant environment. Cellular responses to fluid-induced stress differ for steady and oscillating flows (Jacobs et al., 1998). A steady flow may constantly circulate oxygen, ions, growth factors and nutrients to exposed cells, while an oscillating flow may not provide constant supply or removal of molecules. Oscillating flows in finite domains also exhibit complex temporal redistribution of momentum, compounded or dominated by inertial and edge effects. Currently utilized cone-and-plate and parallel-plate Poiseuille flow loaders (Brown, 2000) could generate uniform synchronous oscillating shear by alternating flow direction, but without the stress non-uniformity, pressure distribution, frequency response, or lateral velocity field seen in synovial joint physiology. There is evidence among joint disorders that the non-uniform shear stress may cause biologically significant outcomes. For instance, osteoarthritis, a common chronic and disabling disease in the elderly, is linked to alteration of joint surface congruity.

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Our study suggests the need for realistic loader geometry with relative motion of opposing articular surfaces, and for accounting of the significant effects of frequency and domain size.

We developed a novel shear loader, an oscillating Couette-flow loader, for studying shear responses of joint tissues (Sun et al., 2003). In this loader, sliding motion of a finite mobile surface parallel to a stationary surface closely resembles a physiologically relevant flow in synovium. The present study aimed at predicting the shear stress and flow velocity in an oscillating flow induced by the loader. A second aim was to develop progressively more realistic simulations of synovial joint mechanics. Oscillating flow between a pair of unbounded plates has been applied to other problems, but no studies are known for flow induced by an oscillating plate with a finite width. Here, we built the analytical and numerical models for the oscillating Couette flow, and evaluated the effects of finite plate dimension on shear distribution.

We first used an analytical model to estimate the magnitude of shear stress on cultured cells immobilized under an oscillating flow (Fig. 1). In the model, the unbounded bottom plate remains stationary, while the unbounded upper plate oscillates sinusoidally at frequency $f = \omega/(2\pi)$ Hz with velocity amplitude U cm/s, traveling each way $2U/\omega$ cm. If the frequency is very low or the gap (depth h cm) is very small, viscous forces dominate inertial forces in the fluid (density ρ g/cm^3, viscosity μ poise), which everywhere moves (and stops) in phase with the top plate. The velocity profile is then always linear, and the instantaneous shear stress τ dyn/cm^2 is uniform with amplitude (maximum value during a cycle) $\mu U/h$, similar to a steady flow. Most experimentalists using various oscillating flows have made this quasi-steady assumption, but it fails when the frequency or the gap is large, and a general solution is required.

For infinite aspect ratio (unbounded plates), the flow field is a function only of height, y, and time, t. The governing equation for the flow velocity $u(y,t)$, in non-dimensional form, is

$$\frac{\partial(u/U)}{\partial(t/T)} = \frac{1}{Re_\omega}\frac{\partial^2(u/U)}{\partial(y/h)^2} \tag{1}$$

introducing $Re_\omega = \rho h^2/T\mu$, the oscillatory Reynolds number, where $T = 2\pi/\omega$, the cycle time period.

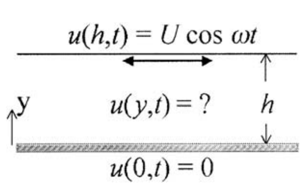

Fig. 1. Schematic diagram of oscillating Couette flow illustrating geometric parameters and boundary conditions.

Remarkably, Re_ω, the ratio of inertial to viscous forces, is independent of U. An analytical solution for $u(y,t)$ may be obtained (Halpern and Frenkel, 2001), from which the shear stress amplitude at any location is

$$|\tau| = \left|\mu\frac{\partial u}{\partial y}\right| = \mu\frac{U}{h}|A|, \quad \text{where } A = \frac{\sqrt{\mathrm{i}\varpi}\cosh{(y/h)}\sqrt{\mathrm{i}\varpi}}{\sinh\sqrt{\mathrm{i}\varpi}},$$
$$\varpi = 2\pi Re_\omega. \tag{2}$$

The shear stress amplitude amplification factor, $A(Re_\omega)$, for the oscillating and stationary plates (Fig. 2), verifies that for low-frequency oscillation the shear stress approaches the viscous limit, $|\tau| \approx \mu U/h$. When Re_ω is large, the fluid's inertia causes it to generally lag behind the plate motion. For sufficiently high frequency, low viscosity, or large gap, fluid away from the oscillating plate hardly moves, and the cells on the fixed plate experience negligible shear stress. With 10 Hz oscillation in a 0.2 cm gap of typical aqueous solution with $\mu = 0.016$ ps, $\rho = 1$ g/ml, ($Re_\omega = 25$), the moving plate experiences over 12 times as much stress, while the cell-laden fixed plate receives less than 100 of the corresponding viscous limit values. For very high Re_ω, all the action occurs near the upper plate, and the relevance of h and Re_ω disappears.

In the in vitro experiment and in real animal and human joints the oscillating plate is necessarily finite. The finite plate span allows convection normal to the plate and produces a non-uniform shear stress distribution on the lower plate. In a computational analysis, an incompressible laminar flow model was developed in which a thin plate (length 3 cm) was placed 0.1 cm above a stationary cell culture surface, and oscillated sinusoidally at 1 Hz with end-to-end amplitude of 2 cm. The computational mesh was given over a large domain with maximum refinement near the plate. To evaluate numerical errors, a reference computation was conducted for an unbounded plate. The calculations were

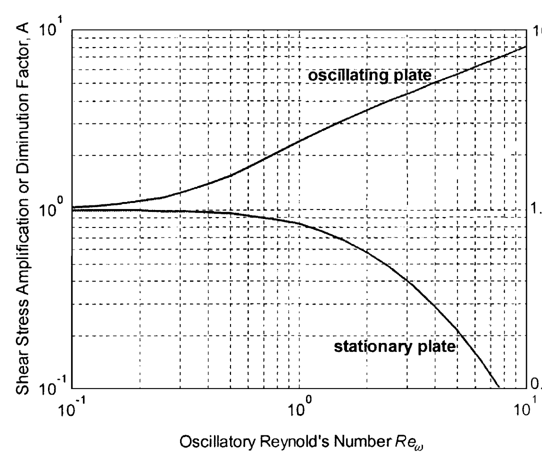

Fig. 2. Shear stress amplification or diminution factor for the unbounded oscillating and stationary plates as a function of oscillatory Reynolds number, Re_ω.

performed using the STAR-CD® code, a general purpose, finite-volume algorithm, operating on a versatile unstructured grid system. Details of the computational method and its extension to curved surfaces with menisci in the knee joint are given elsewhere (Pekkan et al., 2003).

The simulation provided time-varying flow field data for the 2D configuration. The computed velocities for the unbounded plate were in good agreement (within 5%) with analytical prediction. The plate-parallel velocity component at mid-channel depth was evaluated at two locations for the finite plate case (Fig. 3a): directly below the mean position of the plate edge (v_1 at $x=-1.5$ cm), and below the mean plate center position (v_2 at $x=0$ cm). At v_2, the amplitude was about 20% lower for the finite plate, but with similar velocity profile as the unbounded plate. More dramatically, the profile at v_1 was distinctly different: the velocity diminished and restored with the departure and return, respectively, of the moving plate above v_1. The acceleration at the leading edge of the plate generated a stress peak moving with the plate (Fig. 3b) that exceeded twice the stress generated by the unbounded plate, while a milder stress peak tailed the trailing edge.

The most relevant measure of mechanical stimuli to cells is probably the shear stress amplitude at each location on the stationary plate, regardless of the time phase when it peaks there (Fig. 3c). The unbounded plate subjects all the locations to a shear stress amplitude of 0.09 Pa, while the finite plate produces a nearly uniform stress amplitude of about 0.07 Pa in a 1.2 cm central region, slightly wider than the region [−0.5, 0.5 cm] always covered by it. Immediately outside this 'central uniform' region, the maximum shear stress rises sharply to over twice this level in spots before dropping asymptotically to zero at further distances. The peak stress predicted was nearly 0.20 Pa at locations about 2 cm from the center, with non-sinusoidal periodic variation.

The analytical and computational analysis facilitates selection of mechanical parameters for cell culture experiments, and the estimation of conditions such as velocity, shear stress, and stress variations. While computational analysis can predict location-dependent stresses, it is not feasible to do so for all the experimental slider geometries mimicking the wide range of animal locomotion dynamics. Instead, we apply the analytical result, but illustrate the inference of corrected stresses for the experiment, based on proportional comparison of analytical and computational results for the specific geometry presented earlier. For example, an oscillating Couette-flow shear loader was applied to examine oscillatory shear effects on gene expression of MMP-1 in MH7A synovial cells (Sun and Yokota, 2001). A 1 cm × 1 cm slider in the loader was positioned parallel to the culture plate with a 0.2 cm fluid gap, and

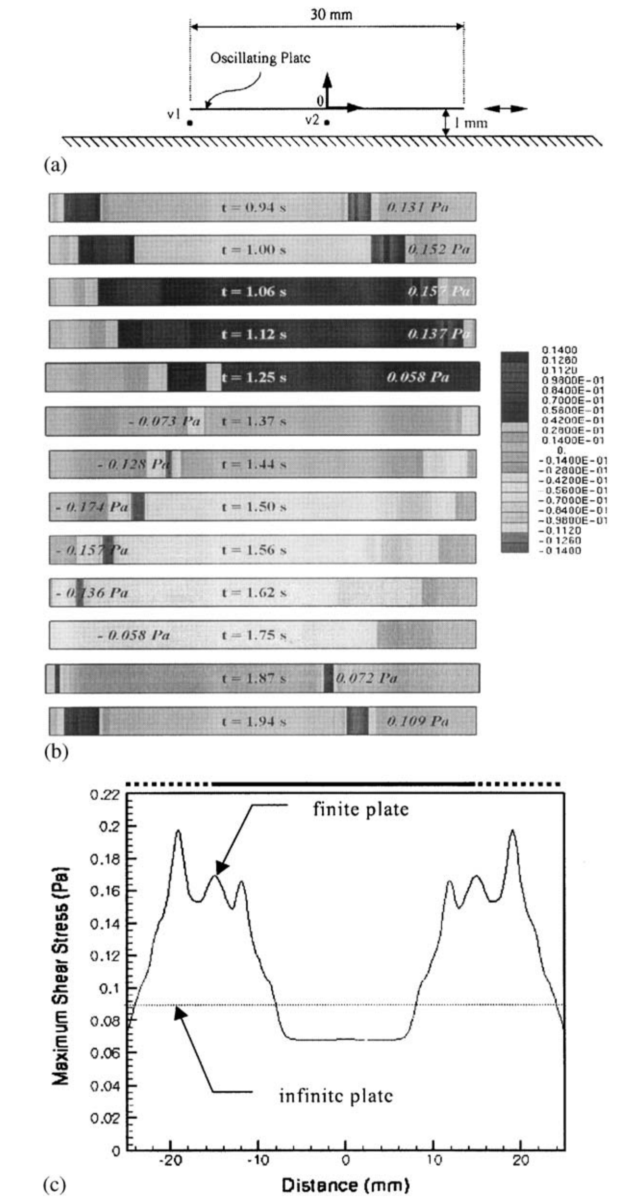

Fig. 3. Shear stress distribution predicted by numerical simulation: (a) A plate was initially positioned over [−1.5, 1.5 cm]. Fluid velocities evaluated at the mid-height distance at $\mathbf{v}_1$, $x=-1.5$ cm, and $\mathbf{v}_2$, $x=0$ cm. (b) Shear stress distributions (in Pa) on the [−2.5, 2.5 cm] region of the stationary wall for a finite oscillating plate. At $t=0.94$ sec, plate movement is to the right ($+x$ direction) and (c) temporal maximum shear stress over [−2.5, 2.5 cm] at the stationary plate.

oscillated sinusoidally at 1 Hz with end-to-end amplitude of 1 cm (3.14 cm/s velocity amplitude). We estimated this slider to generate peak shear stress of approximately 1 dyn/cm^2 on the fixed plate, at locations about 0.75 cm from the center point. We took the shear stress at the center to be roughly 0.4 dyn/cm^2, reduced from the unbounded-plate value by the same proportion as the computed case.

In summary, the current study considering non-linear and multi-dimensional effects revealed that (i) compared to shear induced by an unbounded moving plate, a finite moving plate generated about 20% lower shear stress amplitude in a central uniform region, and (ii) finite span of the moving plate induced two transient stress peaks below the leading and trailing edges of the plate. It also verified that, for values of oscillatory Reynolds number much greater than unity, simple viscous theory overpredicts shear stress on the stationary plate. For simulating the articular joint shear stress, the described loader may offer an improved method closer to synovial fluid motion, as it does not utilize constant flow or fixed rotation direction. Artifacts associated with pressure-driven flow changes in Poiseuille flow, such as sudden spikes or formation of air cavities are avoided. Further, by using curved and/or non-congruous surfaces and non-linear motion, the loader can potentially simulate shear induced by relative motion of complex articular surfaces in joints. It is important to accurately characterize in vitro experimental shear stress, including regimes of achieved loader motion outside common physiological experience. Loader design and cell placement must and can feasibly anticipate the finite-width and frequency effects. In conclusion, the described oscillating Couette-flow loader can generate physiologically relevant oscillatory shear that is well characterized by the given models.

Acknowledgements

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