Unique Cell Shearing Device for Studying Endothelial Mechanobiology Glycation Endproducts

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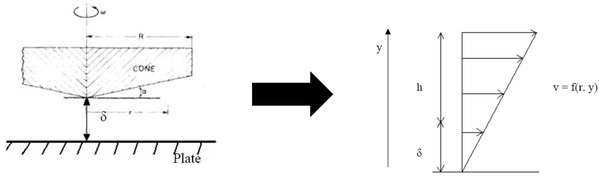
Introduction

The endothelium plays an active role in maintaining the delicate balance between inflammatory processes underlying atherogenesis and anti-inflammatory mechanisms, and is sensitive to a multitude of physiological stimuli, including hemodynamic forces. The strong correlation between local flow characteristics in arteries and focal susceptibility to atherosclerosis is well established. Sites of disturbed flow (DF) are considered to be susceptible to lesion formation, whereas sites of undisturbed flow (UF) are relatively resistant. Although atherosclerosis is recognized as a multifactorial disease, it is currently not well understood how multiple risk factors act synergistically in the context of differential arterial hemodynamics to affect the molecular processes leading to focal lesion formation. We have developed a device capable of subjecting endothelial cells (EC) to a multitude of shear stress (SS) waveforms and flow regimes. Our cell shearing device is based off the principles behind a cone and plate viscometer, and is controlled by a precise microstep motor. By controlling parameters such as the angle of the cone, angular velocity, and fluid viscosity, we are able to achieve a low Reynolds number, ensuring uniform, laminar flow within the chamber. We are able to expose EC to multiple flow profiles with this device, including steady SS of different magnitudes, oscillatory and pulsatile SS, and more complex physiological SS waveforms that mimic *in vivo* blood flow. The device is equipped with a precise temperature control system, allowing for long-term studies to take place. Furthermore, fluid can be circulated during operation, allowing for cell growth media to be replenished while waste is removed, as well as the addition of pharmacological agents and treatments. The features of this device make it ideal for a wide range of flow applications. It has proven to be a valuable tool in elucidating endothelial mechanobiology, and determining the role of flow and SS in atherosclerosis.

Fluid Mechanics Model

•Fluid mechanics model is based from principles of cone and plate viscometer
  •Adapted from Bussolari et al., 1982[1]



r = radius of cone
h = height along the y axis
v = fluid velocity
ω = the angular velocity of the cone
δ = height from plate surface to cone tip
α= angle of cone
μ = dynamic viscosity
υ = kinematic viscosity
τ_w = wall shear stress

$$v(y) = v_{\max}\frac{y}{h+\delta} = \frac{wry}{h+\delta}$$

$$\tan\alpha \approx \alpha = \frac{h}{r} \Rightarrow h = r\alpha \quad \text{(for small } \alpha\text{)}$$

$$v_{\max} = \omega r$$

$$v(y) = \frac{\omega ry}{r\alpha} = \frac{\omega y}{\alpha}$$

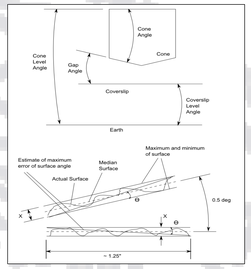
$$\tau_w = \mu\frac{\partial v}{\partial y} = \frac{\mu\omega}{\alpha+\frac{\delta}{r}} = \frac{\mu\omega r}{r\alpha+\delta} \Rightarrow \tau_w = \frac{\mu\omega}{\alpha} \quad \text{(assuming } \delta \to 0\text{)}$$

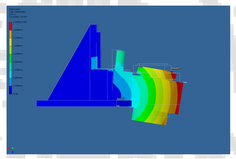
•Dimensionless parameter R is the ratio between the centrifugal and viscous forces
•R<1 ⇒ velocity of fluid beneath the cone is azimuthal ⇒ meaning u (z, r) = 0
•SS equation is valid at R<1 the fluid streamlines are perfectly concentric circles

$$R = \frac{r^2\omega\alpha^2}{12\upsilon}$$

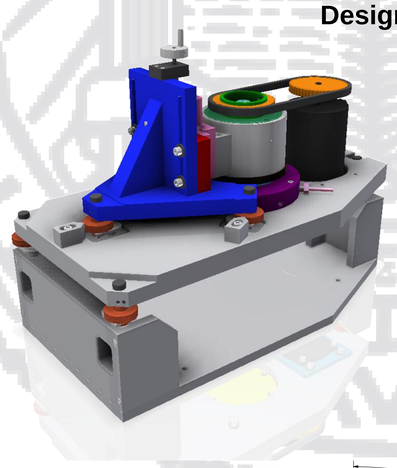
Criteria and Constraints

•The cone gap height is set to 20 +/- 10 microns with a precision depth gage
•The angle of the cone relative to the plate is set to 0.5 deg +/- 0.05 degs with a precision level
•16 RMS finish on the cone's surface
•Underside visualization of the flow patterns
•Temperature of the media is 37 +/- 2 degrees Celsius
•Media is circulated to prevent drying
•Coverslip and cell growth area has a diameter of 75 mm
•Coverslips are removable
•Fine (0.01"/rot) and coarse (0.06"/rot) height adjustment height adjustment
•Runs constantly for up to 72 hrs
•Reproduces realistic blood velocity patterns (up to 40 rad/s)
•Applies shears stresses with laminar flow from 0 to 100 dynes/cm²

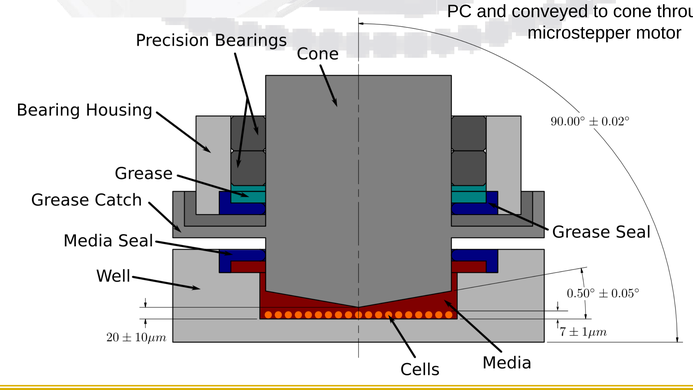




Design



•Current design allows for EC to be cultured on disposable collagen treated substrates
  •Substrates are placed in chamber from bottom
    •Eliminates affects of variation in substrate thickness
•Two set of leveling mechanism ensures cone is completely perpendicular to cell substrate
•Depth gauge and access through center of cone allows gap height to be set an accuracy of 10 μm
•Cell chamber is kept within 0.5 °C of 37°C
•Rotating cone induces precisely defined shear stress on EC monolayer
  •Shear stress program is input into a PC and conveyed to cone through microstepper motor



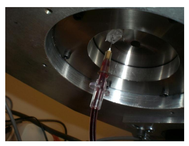
Features of Device

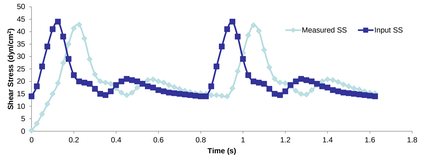
•Enclosed in clear polycarbonate case
  •HEPA filter-covered fans create positive internal air pressure
    •Ensure sterile environment
  •PID controller, thermocouple, heaters, fans
    •provide sensitive, accurate temperature control
•Microstepper motor
  •25,000 steps/revolution
  •Simulate multitude of shear stress profiles
    •Wide range of high/low steady flow, pulsatile flow, oscillatory flow, complex arterial shear stress waveforms
•Access ports
  •Media exchange
  •Introduce pharmacological agents

Analysis and Validation

•Flow visualization was performed to ensure laminar flow
  •Dye is injected into flow field while running
  •Dye forms concentric circle—showing flow is laminar, fluid velocity is one-dimensional

• Encoder used to measure angular velocity of cone
•A blood flow waveform acquired by ultrasound from the distal internal carotid artery of a normal human subject[2] was programmed into the cell shearing device
•Angular velocity was measured directly from the motor
  •Values of shear stress were calculated at the plate surface





Summary and Future Direction

•A Cell Shearing Device was constructed that satisfied all design constraints and criteria
•Accuracy of the device in producing laminar flow , and desired input shear stress waveforms was assessed and validated
•Future analyses will determine and confirm the biocompatibility of the device and ensure no cytotoxicity
•Device will then be employed as a powerful tool to examine the cellular response to physiological flow/ shear stress in a wide range of applications

References

1. Bussolari et al. Apparatus for subjecting living cells to fluid shear stress. *Rev. Sci. Instrum*, 1982, 53(12).
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3. Blackman et al. A new in vitro model to evaluate differential responses of endothelial cells to simulated arterial shear stress waveforms. *J Biomech Eng.*, 2002 Aug;124(4):397-407.