Why is Laminar Fluid Shear Stress Important?

Cells *in vivo* are exposed to shear stress when liquids (extracellular fluid, blood, lymph, urinary filtrate, etc) move across their surface. This fluid shear stress potently affects cellular structure and function [1]. As we strive to engineer tissue constructs and develop physiologic model systems, it is becoming increasingly apparent that fluid shear stress is also a critical component of *in vitro* cultures. For example, fluid shear stress drives the differentiation of stem cells to diverse lineages [2-12]. Proximal tubule kidney cells maintain their brush border and transporter functions and liver cells maintain critical drug transporters only when cultured in the presence of fluid shear stress [13-18].

Fluid shear *in vivo* is typically laminar, meaning that the fluid flows smoothly parallel to the surface of the cell. However turbulent or chaotic flow can be seen in certain areas such as the bifurcation of blood vessels [19, 20]. Laminar and turbulent flows have very different effects with turbulence often leading to apoptotic and necrotic forms of cell death *in vivo* and *in vitro* [9, 21, 22].

Many laboratory studies of fluid shear stress use parallel plates, or cone and plate systems that moved fluid across the surface of a 2D monolayer of cells [22]. The advantage of controllable laminar fluid shear stress in these systems is offset by their requirement for planar cell culture. 3D cellular cultures are more physiologic and are a crucial requirement in tissue engineered constructs [23]. Studies also suggest that cells in a 3D configuration are more sensitive than cells in a 2D culture to fluid shear stress which increases the *in vivo* applicability of data from 3D *in vitro* models [24, 25].

In stirred bioreactors, fluid shear stress is not laminar or uniform. Paddles or impellers must spin fast enough to keep cells, microcarriers, or spheroids in suspension. But this leads to impacts between cells and the impellers, impacts between microcarriers/spheroids and themselves, and shear in turbulent eddies, all of which are injurious or lethal, particularly for mammalian cells [26-29]. Rocking or swirling in low adherence dishes or bags allows for cells to organize into 3D constructs or spheroids, but the fluid shear forces are neither laminar nor uniform.

Rotating suspension vessels, such as those marketed by our company Cell Spinpod [30], keeps 3D particles in suspension by rotating them at a rate that offsets their sedimentation under the influence of gravity. Rotating suspension culture can be used with non-adherent cells or organisms, 3D spheroids or organelles, or even cells on a microcarrier for an 'anchorage-optional alternative'. The fluid shear stress in a zero headspace (e.g. no air bubble) rotating suspension culture is laminar and uniform, but is also relatively fixed in intensity at approximately 0.05 dynes/cm^2. Stepwise increasing

the rate of rotation, merely moves the cells into an ever larger annular path until they impact the walls of the vessel.

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