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Hypothesis and Evidence: Potential Cellular Damage Induced by Pipetting Flow

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Abstract:

Fluid shear stress, a critical mechanical factor in the cellular microenvironment, regulates cell morphology, function, gene expression, and signaling pathways, making it a research focus in life sciences and biomedical engineering. Responses to shear stress vary significantly across cell types (e.g., vascular endothelial cells, chondrocytes, immune cells, and tumor cells), with force magnitude, duration, and flow patterns (laminar vs. turbulent) eliciting distinct biological effects. In the cardiovascular system, shear stress modulates atherosclerotic plaque stability by regulating endoplasmic reticulum stress proteins (e.g., GRP78 and CHOP). In cartilage, shear stress synergizes with inflammatory factors (e.g., TNF-α) to influence cell phenotype maintenance and extracellular matrix synthesis. Under pathological conditions (e.g., thrombosis), abnormally high shear stress (>1000 dyne/cm²) directly causes cell detachment and damage. Notably, cells exhibit "mechanical memory," whereby transient exposure to low shear stress triggers long-term changes in endothelial cell traction forces, altering alignment and function. Given that in vitro manipulations (e.g., pipetting) introduce uncontrolled fluid stress—compromising cell viability and experimental reproducibility—microfluidic technologies enabling precise shear stress control and standardization offer a vital solution to optimize cell-based assays and enhance data reliability.

Keywords: Cell-based assays; Pipetting; Experimental reproducibility; Standardization

1. Background:

Cell-based experiments are fundamental in life sciences, utilizing diverse samples (e.g., tumor cells, fibroblasts, immune cells) with varying sensitivity and resilience. For instance, pipetting-induced mechanical dissociation reduces immune synapse formation in immune cells, and newly revived cardiomyocytes require cytoskeletal reorganization. Such mechanically sensitive cells necessitate gentle handling.

Current pipetting techniques involve intricate operational details often overlooked by users, leading to inconsistencies that compromise experimental accuracy and reliability. Critically, pipetting may exert adverse effects on cells, disrupting physiological states and confounding conclusions. Mechanically, pipetting generates substantial stress on cells; studies indicate that routine pipetting produces shear stresses of 100–1000 dyne/cm², sufficient to cause immediate mechanical damage. Additionally, mechanical stress exposure exhibits cumulative effects: short-term shear stress may induce long-term functional alterations via cellular mechanical memory mechanisms. To systematically evaluate pipetting-induced cellular impacts, we reviewed existing literature and calculated stress parameters. Due to limited studies in this niche, we prioritized fluid dynamics as a key analytical lens to identify risks and mechanistic insights. This review summarizes evidence-based strategies to enhance experimental rigor.

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2. Primary Fluid Dynamics Force Affecting Cells: Shear Stress

Shear stress, alongside compressive and circumferential stresses, profoundly influences cellular morphology, growth, repair, and function, with shear stress being the most prominent factor.

In straight arterial segments, laminar blood flow generates high shear stress. At vascular bifurcations, however, flow transitions from high- to low-shear zones promote lipid deposition and thrombosis. Shear stress critically regulates atherosclerotic plaque stability by modulating endoplasmic reticulum stress proteins (e.g., GRP78 and CHOP). Hemodynamic factors (e.g., viscosity, flow velocity) collectively alter endothelial cell phenotypes at cellular, molecular, and genetic levels, driving plaque pathogenesis [1]. While computational fluid dynamics addresses vascular complexity, this field remains ancillary to our focus. Pathologically, supraphysiological shear stress (>1000 dyne/cm²) induces cell damage and detachment. Endothelial cells perceive hemodynamic signals (shear stress, pressure, strain) via membrane receptors, activating biochemical cascades that regulate transcription, replication, and protein expression [2]. High shear stress stabilizes carotid plaques by targeting specific proteins—a principle potentially applicable to pipetting. Analogously, transient high shear stress (>1000 dyne/cm²) during pipetting exceeds cellular tolerance thresholds, triggering similar damage mechanisms, thereby contributing to poor reproducibility.

In cartilage, fluid shear stress modulates chondrocyte hypertrophy, dedifferentiation, and apoptosis. Synergy with TNF-α disrupts phenotype maintenance and matrix synthesis [3]. Shear stress also accelerates intervertebral disc degeneration via effects on cartilaginous endplates, annulus fibrosus, and nucleus pulposus [4]. Its bidirectional effects—beneficial at low levels but detrimental at high levels—extend to cancer cells (e.g., promoting proliferation/metastasis in hepatocellular and breast cancer cells) [5]. In neural contexts, cerebrospinal fluid-derived shear stress guides axonal growth; low-level stress promotes directional neurite extension [6], while high stress inhibits it. Immune cells employ mechanosensitive proteins to transduce mechanical cues into activation signals. Critically, mechanical memory—mediated by the YAP/TAZ pathway [7]—enables sustained adaptations to transient shear stress exposures, underscoring the need to control cumulative stress during pipetting.

3. Conclusion

Fluid dynamics, particularly shear stress, directly shapes cellular morphology and function while influencing disease progression. During pipetting, fluidic forces are unavoidable and often unregulated. Microfluidic technologies standardizing shear stress (<5 dyne/cm²) mitigate uncontrolled fluid stress, enhancing cell viability and data reproducibility. Devices like microfluidic pipetting adaptors (e.g., Beijing Ennovate Joy Bio-Tech Co.) homogenize flow rates, minimizing experimental variability. For cell-containing samples, integrating fluid dynamics considerations is essential to reduce errors and improve scientific rigor.

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