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Influences of Pipetting Fluid Dynamics on Cell Status

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Abstract:

In cellular experiment, pipetting is one of the most common basic procedures. Hydrodynamic factors during pipetting, such as flow rate, shear force, and pressure changes, can exert multiple effects on cell status (including many adverse impacts). This article comprehensively elaborates on the relevant principles of pipetting hydrodynamics, as well as its influences on cell morphology, viability, proliferation, differentiation and other states. It also explores methods that can effectively reduce adverse effects and optimize pipetting operations, aiming to provide a theoretical basis for the standardization and precision of pipetting in cell experiments, and promote the improvement of the accuracy and reliability of cell experiments.

Keywords: Microplastics, Cardiovascular Toxicity, Biomarkers, Detection Methods

1. Background:

Cellular experiments play a crucial role in numerous fields such as biology and medicine, and the accuracy of their results directly relates to the reliability of research conclusions. Pipetting, as a frequently performed operation in cell experiments, may seem simple, but it actually has a non-negligible impact on cell status. Traditional concepts have mostly focused on the accuracy of pipetting to ensure the precise amounts of reagents, cell suspensions, etc., in the experimental system. However, in recent years, studies have gradually revealed that the hydrodynamic characteristics involved in the pipetting process can significantly affect the microenvironment where cells are located, thereby changing cell status such as morphology, viability, proliferation ability, and differentiation direction. An increasing number of researchers conducting cell experiments have also paid attention to the "impact of pipetting hydrodynamics on cell status". A thorough understanding of the influence of pipetting hydrodynamics on cell status is of great significance for optimizing cell experiment operations and improving experiment quality.

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2. Hydrodynamics-related concepts involved in pipetting operations

2.1 Flow rate

Flow rate refers to the distance a fluid travels per unit time. In pipetting operations, the flow rate depends on the type of pipette, the set volume, and the speed at which the liquid is pushed. Different types of pipettes, such as manual pipettes and electric pipettes, differ in terms of flow rate control. The flow rate of manual pipettes largely depends on the experience and technique of the operator, while electric pipettes can achieve more stable and repeatable flow rate adjustment through precise motor control.

In cell experiments, pipetting flow rate is of great significance. During cell seeding, an appropriate flow rate can ensure that cells are evenly distributed in the culture vessel. If the flow rate is too fast, cells may over-aggregate in local areas, leading to an uneven cell growth environment and affecting normal cell growth and metabolism. In operations such as replacing cell culture medium, a suitable flow rate can ensure that the old medium is fully removed while avoiding excessive impact on the cells.

2.2 Shear force

Shear force is the mutual force generated due to the velocity difference between fluid layers when a fluid flows. During pipetting, shear force is generated when

the liquid flows inside the pipette tip and when it is ejected from the tip. The magnitude of shear force is closely related to factors such as flow rate, liquid viscosity, and the inner diameter of the tip. Generally speaking, the faster the flow rate, the higher the liquid viscosity, and the smaller the tip inner diameter, the greater the shear force generated.

Cells are sensitive to shear force, and different types of cells have different tolerance levels to shear force. For example, blood cells have adapted to a certain range of shear force in the blood circulation, while some adherent cells, such as fibroblasts, have relatively low tolerance to shear force. When the shear force on cells exceeds their tolerance range, it may cause damage to the cell membrane, changes in the cytoskeleton structure, etc., thereby affecting the normal function of cells.

2.3 Pressure

There are various pressure changes during pipetting, including the pushing pressure generated inside the pipette, the static pressure of the liquid in the tip, and the pressure generated when the liquid comes into contact with cells. The pushing pressure is provided by the piston or pneumatic device of the pipette to overcome the resistance of the liquid and realize the aspiration and discharge of the liquid. Static pressure is related to the height of the liquid column and the liquid density.

The impact of pressure on cells is more complex. Excessively high pushing pressure may cause the liquid to eject too fast, generating excessive shear force and impact force, which can damage cells. Changes in static pressure may affect the material exchange and osmotic pressure balance inside and outside the cells. In operations such as cell transfection, improper pressure control will affect the interaction between transfection reagents and cells, reducing transfection efficiency.

3. The impact of hydrodynamics on cell status

Factors such as flow rate, shear force, and pressure in pipetting hydrodynamics can significantly affect cell morphology, viability, proliferation and differentiation, migration, and metabolism by regulating cell gene expression. For example, fluid shear force can promote the activation and nuclear translocation of ERK5 in osteoblasts through the NO/cGMP/PKG signaling pathway [1]; mechanical pressure promotes the expression of α-SMA protein in LX-2 cells, promotes the expression of COL1A1 mRNA, and inhibits the expression of TGFB1 mRNA[2]; fluid shear force can stimulate the activation of ERK5 in osteoblasts, and the activated ERK5 further plays a regulatory role in the proliferation, differentiation, and apoptosis of osteoblasts[3]. Most existing studies focus on the regulatory role of hydrodynamic characteristics on cell status in vivo or in simulated in vivo environments. It is worth noting that such changes in cell status caused by hydrodynamics may also occur during the pipetting operation in cell experiments. Therefore, the conclusions in these studies regarding the association between hydrodynamics and cell status can provide an important reference for this study to explore the potential impact of pipetting operations on experimental results.

3.1 Impact on cell morphology

Cell morphology is an intuitive manifestation of cell status, and factors such as shear force and pressure in pipetting hydrodynamics have a significant impact on cell morphology.Shear force can change the morphology and skeleton of endothelial cells[4]. For adherent cells, phenomena such as cells detaching from the culture surface and changes in cell spreading morphology may occur. Studies have shown that human dermal microvascular endothelial cells and cell nuclei have corresponding morphological responses to steady-state, laminar, and unidirectional fluid shear stress[5]; a study constructed a vascular endothelial cell injury model and found that fluid shear force causes damage to the adhesive junctions between vascular endothelial cells through the PKC α-P120ctn-NF-B signaling pathway, leading to vascular endothelial injury[6].

Pressure changes also affect cell morphology, which in myoblasts is manifested as cells becoming flattened and larger[7]; in osteoclasts, it is manifested as changing from irregular in 2D images and flat in 3D images to round in 2D images and arched in 3D images (changing from a non-resorptive state to a resorptive state)[8]. When the pressure generated during pipetting is uneven, cells may be subjected to local compression or stretching, resulting in distorted cell morphology. This change in morphology may further affect cell functions, such as cell migration ability and material transport ability.

3.2 Impact on cell viability

Cell viability reflects the viability and metabolic activity of cells, and pipetting hydrodynamic factors have a direct or indirect impact on cell viability. The rheological properties of cells are closely related to the cytoskeleton-containing cell membrane[9]. Excessive shear force and pressure may damage the integrity of the cell membrane, causing the leakage of ions and small molecular substances in the cell, thereby affecting the normal metabolism of the cell and reducing cell viability. A study found that in vitro continuous pressure can stimulate the increase in the expression of MMP-9 and TRAP in mature osteoclasts, and these two proteins are indicators for testing osteoclast activity and bone resorption function[10].

A large number of studies have found that hydrodynamic factors induce cell apoptosis through a series of signaling pathways: fluid shear force promotes glomerular endothelial cell apoptosis by activating Piezo 1 protein[11]; fluid shear stress-mediated Piezo1 alleviates osteocyte apoptosis by activating

the PI3K/Akt pathway[12]; continuous static pressure promotes retinal ganglion cell apoptosis through the TRPV4 signaling pathway[13]; within a small range of hydrostatic pressure, it may inhibit the apoptosis of rat condylar chondrocytes, and when the pressure exceeds a certain limit, it may promote the apoptosis of chondrocytes[14].

3.3 Impact on cell proliferation

Cell proliferation is an important process of cell life activities, and pipetting hydrodynamic factors can affect cell proliferation through multiple pathways. In recent years, studies have confirmed that direct compressive force, hydrostatic pressure, low shear force, and electromagnetic force can increase the mRNA expression of type II collagen and aggrecan in the extracellular matrix, promoting chondrocyte proliferation; fluid shear stress regulates osteoblast proliferation and apoptosis through the lncRNA TUG1/miR-34a/FGFR1 axis[15]. A study used a pressure loading system and confirmed that under certain conditions, compressive stress can promote cell proliferation[16]. It is reasonable to conjecture that a suitable hydrodynamic environment may provide better material transport conditions for cells, promote the uptake of nutrients and the excretion of metabolic waste, thereby facilitating cell proliferation.

3.4 Impact on cell differentiation

Hydrodynamics plays an important role in the process of cell differentiation. Fluid shear force can promote the osteogenic differentiation of human periodontal ligament cells through the miRNA-132/-PI3K/mTOR signaling pathway, ERK/p38/JNK MAPK signaling pathway, and Wnt/LEF-1 signaling pathway [17]; the co-culture of fluid shear stress and human umbilical vein endothelial cells synergistically promotes the osteogenesis of rat bone marrow mesenchymal stem cells, and this process is mediated by the integrin β1-FAK-ERK signaling pathway[18]; low hydrostatic pressure can promote the chondrogenic differentiation of human embryonic stem cells and human bone marrow stem cells[19]. However, if the hydrodynamic conditions are unstable or inappropriate during pipetting, it may interfere with the differentiation process of stem cells, leading to abnormal differentiation or reduced differentiation efficiency.

3.5 Impact on cell migration

Factors such as flow rate and shear force in pipetting hydrodynamics have an important impact on cell migration and act on physiological and pathological processes such as embryonic development, wound healing, immune response, and tumor metastasis. A study suggested that the Piezo1 ion channel is involved in the regulation of astrocyte activation under simple pressure, ultimately promoting the release of inflammatory factors and cell migration[20]. Normal physiological frequency tensile strain may inhibit the migration of vascular smooth muscle cells by activating vascular phosphorylated heat shock protein 27 to maintain the normal structure and function of blood vessels[21]. Fluid shear force promotes the migration of liver cancer stem cells through the RhoA-YAP1-autophagy pathway[22]. For tumor cells, understanding the impact of pipetting hydrodynamics on their migration is of great significance for studying tumor metastasis mechanisms and developing related treatment strategies.

3.6 Impact on cell metabolism

Cell metabolism is the basis for cells to maintain life activities. Pipetting hydrodynamic factors can affect cell material transport and metabolic microenvironment, thereby affecting cell metabolism. Shear force can regulate the release of nitric oxide, prostaglandins, endothelin, and other cytokines in endothelial cells; studies have found that excessive fluid stimulation can induce the release of pro-inflammatory factors and the degradation of chondrocyte matrix, and high fluid shear force can promote the catabolism of chondrocytes in a short time[23]; excessive mechanical pressure can cause high expression of bone resorption factors in human periodontal ligament cells[24]; in a mouse model of myocardial hypertrophy induced by pressure load, glucose metabolism shows dynamic differential changes[25]. If the pipetting process leads to insufficient supply of nutrients or accumulation of metabolic waste, cell metabolic activities may be inhibited.

4. Strategies to optimize pipetting hydro -dynamics to improve cell status

4.1 Select appropriate pipettes and tips

Different types of pipettes differ in flow rate control, accuracy, etc. When conducting cell-related experiments, appropriate pipettes should be selected according to experimental needs. For example, for experiments that require precise control of flow rate and volume, such as single-cell operations, electric pipettes with high-precision flow rate adjustment functions can be used.

At the same time, the material, inner diameter, and surface treatment of the tip also affect pipetting hydrodynamics. Choosing tips with smooth inner walls, soft materials, and non-toxicity can reduce the resistance during liquid flow and the shear force on cells. In addition, some specially designed tips, such as low-adsorption tips, can reduce the adsorption of cells and liquid components on the tip surface, reducing the impact on experimental results.

4.2 Optimize the pipetting operation process

The operator's technique has a significant impact on pipetting hydrodynamics. By training operators to master correct skills such as pipetting speed, angle, and force, the damage to cells during pipetting can be effectively reduced. When aspirating and dispensing liquid, the movements should be as stable and slow as

possible to avoid violent changes in flow rate and pressure fluctuations. For example, when aspirating cell suspensions, the tip can be immersed to an appropriate depth below the liquid surface and aspirated slowly to avoid inhaling air and generating bubbles. When dispensing liquid, it should also be pushed out slowly to reduce the impact on cells.

4.3 Adopt innovative technologies

Microfluidic technology can precisely control the flow of small volumes of liquid and has unique advantages in cell experiments. Through microfluidic chips, precise manipulation of cells can be achieved while reducing cell damage caused by hydrodynamic factors.

Non-contact pipetting technologies, such as acoustic pipetting and laser-induced pipetting, avoid direct contact between the tip and cells, which can effectively reduce shear force and pollution risks. These advanced pipetting technologies provide new ways to improve cell status, but currently, there may be problems such as high equipment costs and complex operations, which need further optimization and promotion.

Beijing Ennovete Joy Biotechnology Co., Ltd. has provided a variable flow rate pipette tip slow suction piece design and developed a new type of slow suction adapter, which can slow down the pipetting speed of the pipette while ensuring the tip and range remain unchanged; this specially designed slow suction adapter can increase the culture survival rate of NK92 cells by 16.19%, effectively reducing the adverse impact of pipetting operations on cells.

5. Conclusion

Pipetting hydrodynamics has significant multi-dimensional impacts on cell status in cell experiment operations. Every key aspect of cell status, from the maintenance and change of cell morphology to the rise and fall of cell viability, the regulation of proliferation processes, and the guidance of differentiation directions, is closely related to hydrodynamic factors such as flow rate, shear force, and pressure changes during pipetting. Inappropriate pipetting hydrodynamic conditions can lead to abnormal cell morphology, impaired viability, inhibited proliferation, and deviation of differentiation directions from expectations, thereby seriously interfering with the accuracy and reliability of cell experiment results.

By selecting suitable pipettes and tips, precisely controlling pipetting speed, and optimizing pipetting operation steps, the adverse impact of pipetting hydrodynamics on cell status can be effectively reduced, creating a more suitable operating environment for cell experiments. In future cell experiment research, it is necessary to further explore the complex relationship between pipetting hydrodynamics and cell status, and continuously improve pipetting operation specifications to improve the quality of cell experiments and promote the precise development of research in related fields such as biology and medicine.

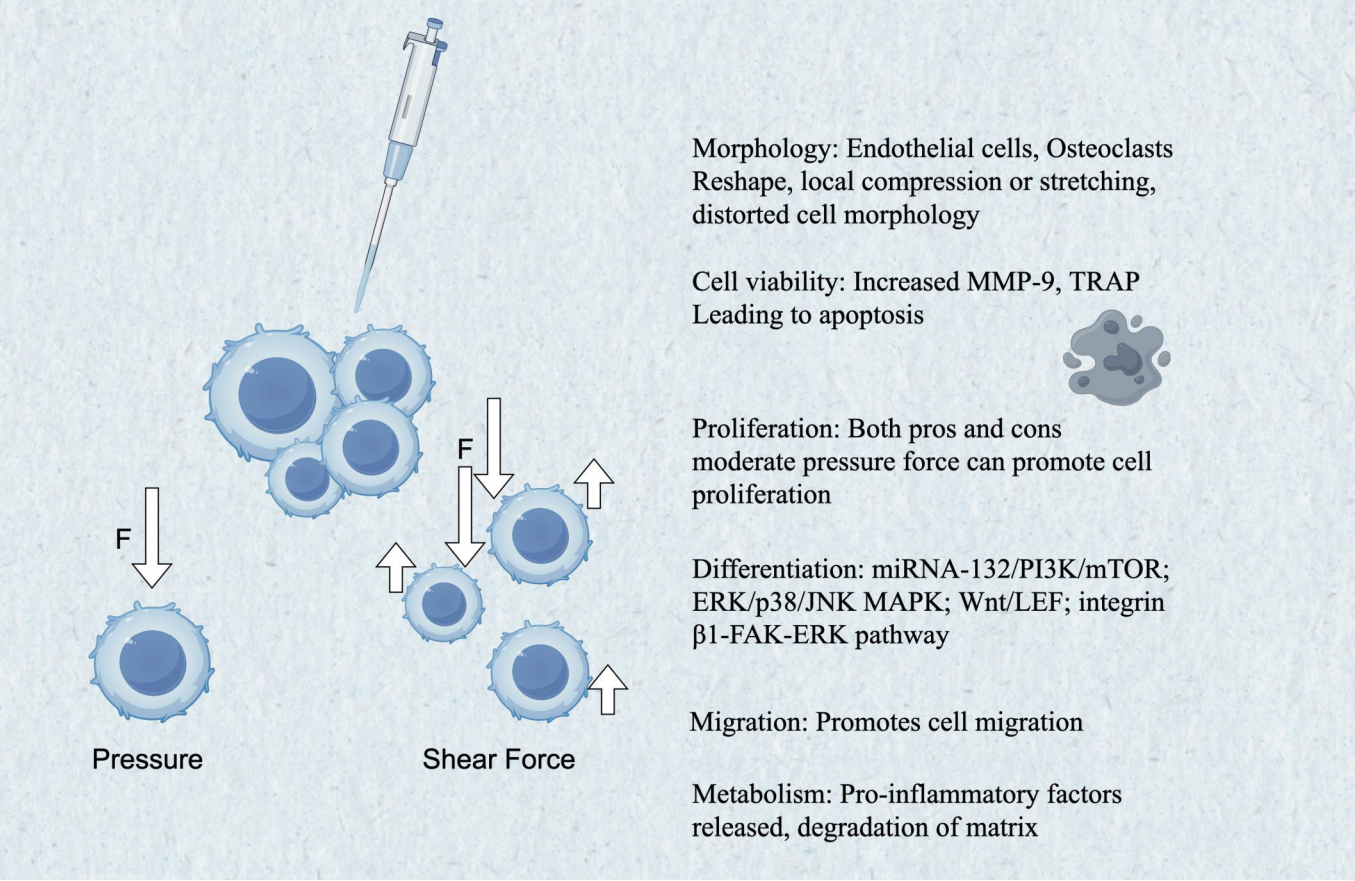

Fig1. Influences of Pipetting Fluid Dynamics on Cell Status

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