Shear-sensitive pumping with peristaltic pumps: gentle on your cells, efficient for your workflow

Controlled levels of shear stress are often used in cell culture experiments to mimic physiological conditions and study the response of cells to mechanical forces. However, unintended exposure of cells in culture to shear stress can impact cell behavior and function, morphology and viability, resulting in reduced growth, altered gene expression and cell death. Changes in gene expression due to shear stress are a particular challenge for drug developers and manufacturers, as these changes can impact recombinant protein production. Given the risk of cell damage and the possible effect on protein production, operating parameters should be designed to ensure that hydrodynamic conditions do not significantly affect cultures. When mitigating shear stress, a key operational factor is choosing the right pumps for adding media and nutrients to culture vessels and for transferring or sampling cultures.

PUMP DESIGN IMPACTS SHEAR STRESS

While various pump designs are used in cell culture applications, peristaltic pumps have become the gold standard for shear-sensitive pumping. Their gentle, low-shear operation minimizes cell damage and suspension agitation, helping maintain experimental integrity and achieve culture objectives. This success is due to the inherent design of peristaltic pumps, which deliver smooth, controlled flow at low speeds, unlike other positive displacement pumps that rely on tightly machined gears or high-speed impellers. It should be noted that not all peristaltic pumps are created equal when it comes to providing maximum protection for shear-sensitive applications, so careful evaluation in selecting the right pump is necessary to ensure optimal performance. Factors such as pump speed and pump head occlusion also impact performance as described below.

EVALUATION OF FACTORS AFFECTING SHEAR FORCES

In the following evaluations, the hemolysis of red blood cells was performed using different pump systems and settings to compare the shear forces generated. Tests were performed by an ANSI-ASQ National Accreditation Board (ANAB)-accredited laboratory using citrated bovine blood and were completed according to ASTM F756-13 (Standard Practice for Assessment of Hemolytic Properties of Materials) and FDA 21 CFR Part 58 (Good Laboratory Practice for Nonclinical Laboratory Studies).

The average hemolytic index, a measure of the percentage of blood cell hemolysis, was calculated at several time points during each 180-min test under different pump systems and settings.

Impact of pump motor speed

The flow rates generated by peristaltic pumps result from the rotation speed of the motor, measured in revolutions per minute (rpm). Peristaltic pumps typically exhibit a linear relationship between flow rate and rpm. As shown in Figure 1, the motor speed of the peristaltic pump is a key factor in minimizing the risk of cell damage and preserving cell viability during the recirculation of media. Higher speeds lead to increased levels of blood cell hemolysis.

The volume of fluid moved per pump revolution is influenced by the tubing diameter, the number of rollers used and the degree of compression (occlusion) applied to the tubing. These factors can also impact cell viability by increasing mechanical stress.

Impact of pump head occlusion settings and geometry

In a peristaltic pump head, occlusion is the degree of compression of the space between the tubing walls when the rollers apply pressure. Full occlusion indicates complete compression, with no space between the walls; partial occlusion indicates incomplete compression, with some space between the walls. In practice, full occlusion generates the highest pressure and flow rates.

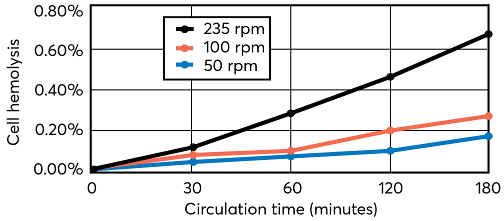

FIGURE 1: Correlation between Masterflex® L/S® Easy-Load® II pump speed and the percentage of blood cell hemolysis.

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In the hemolysis tests, reducing tubing occlusion gave the blood cells more room to move within the tubing, allowing them to shift around the roller's force and thereby avoid excessive compression. This resulted in a lower percentage of hemolysis.

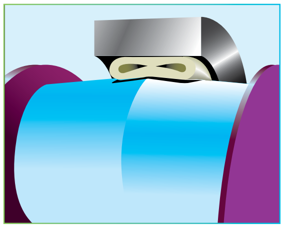

FIGURE 2: Diagram of a convex roller.

FIGURE 4: Masterflex® L/S® peristaltic pump system with two stacked Easy-Load® II pump heads connected via a double-Y tubing assembly.

FIGURE 5: Masterflex® Cytoflow™ pump system.

The occlusion geometry can also affect cell viability. Peristaltic pumps traditionally have flat rollers, which are ideal for most applications, delivering superior dispensing accuracy, maximum pressure capabilities and higher flow rates. However, for shear-sensitive applications, convex rollers perform better. As shown in Figure 2, these rollers function similarly to adjustable-occlusion pumps by creating space for cell movement when the tubing is compressed. Figure 3 compares the effects on hemolysis of using flat versus convex rollers in pumps running at the same speed. Using convex

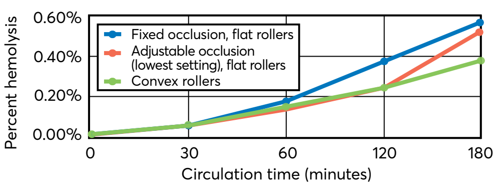

FIGURE 3: Effects of Masterflex® L/S® Easy-Load® II pump head occlusion settings and geometry on blood cell hemolysis.

rollers improved cell viability by 10 - 25% compared to when using a fixed-occlusion flat-roller pump head or an adjustable-occlusion flat-roller pump head at the lowest occlusion setting.

Optimizing cell viability with a flat-roller pump head

The Masterflex® L/S® Easy-Load® II pump head is among the most popular peristaltic pump heads on the market and is commonly used in perfusion, bioreactor and cell suspension applications. This flat-roller pump head can be optimized for cell viability by minimizing the motor speed needed to achieve the targeted flow rate. This can be done using one of two techniques:

- Use two stacked pump heads connected via a double-Y tubing assembly as shown in Figure 4.
- Use the largest-diameter tubing the process will allow.

The Masterflex® L/S® Easy-Load® II pump head can be further optimized by choosing the adjustable-occlusion model and reducing the occlusion as much as process conditions will allow.

Masterflex® Cytoflow™ pump heads were developed specifically for applications with live cell pumping, in which cell viability is essential. They feature convex rollers and have a relatively larger-diameter rotor that enables a higher flow at low motor speeds, making these pump heads ideal for minimizing shear stress and cell damage (Figure 5).

The Masterflex® Cytoflow™ pump head has an extra-large tubing bed and fewer rollers, which enable high flow rates comparable to those generated when using two stacked Easy-Load® II pump heads but without the need for a "Y" pattern in the fluid path. Figure 6 shows how, compared with a single Easy-Load® II pump head, using Cytoflow™ and double-stacked Easy-Load® II pump heads can dramatically limit the speed of the pump when targeting a flow rate of 800 ml/min.

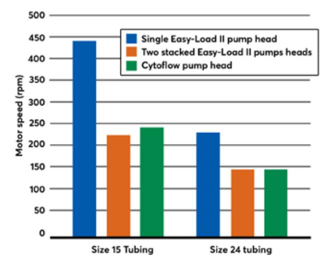

FIGURE 6: Motor speeds required to achieve a flow rate of 800 ml/min using one or two Masterflex® L/S® Easy-Load® II pump heads or a single Masterflex® Cytoflow™ pump head with different tubing sizes.

CONCLUSION

Minimizing shear stress is critical for maintaining cell viability and ensuring the success of cell culture experiments and recombinant protein production. Peristaltic pumps offer a reliable, low-shear solution, but not all models perform equally in shear-sensitive applications. As demonstrated in these evaluations, factors such as pump motor speed, tubing occlusion and roller geometry significantly impact cell viability. Optimizing these parameters, whether through the use of adjustable occlusion settings, larger tubing diameters or advanced pump head designs like the Masterflex® Cytoflow™, can help reduce cell damage while maintaining the necessary flow rates. By carefully selecting and configuring peristaltic pumps, researchers and bioprocess engineers can achieve optimal fluid handling while preserving cell integrity in critical applications.

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