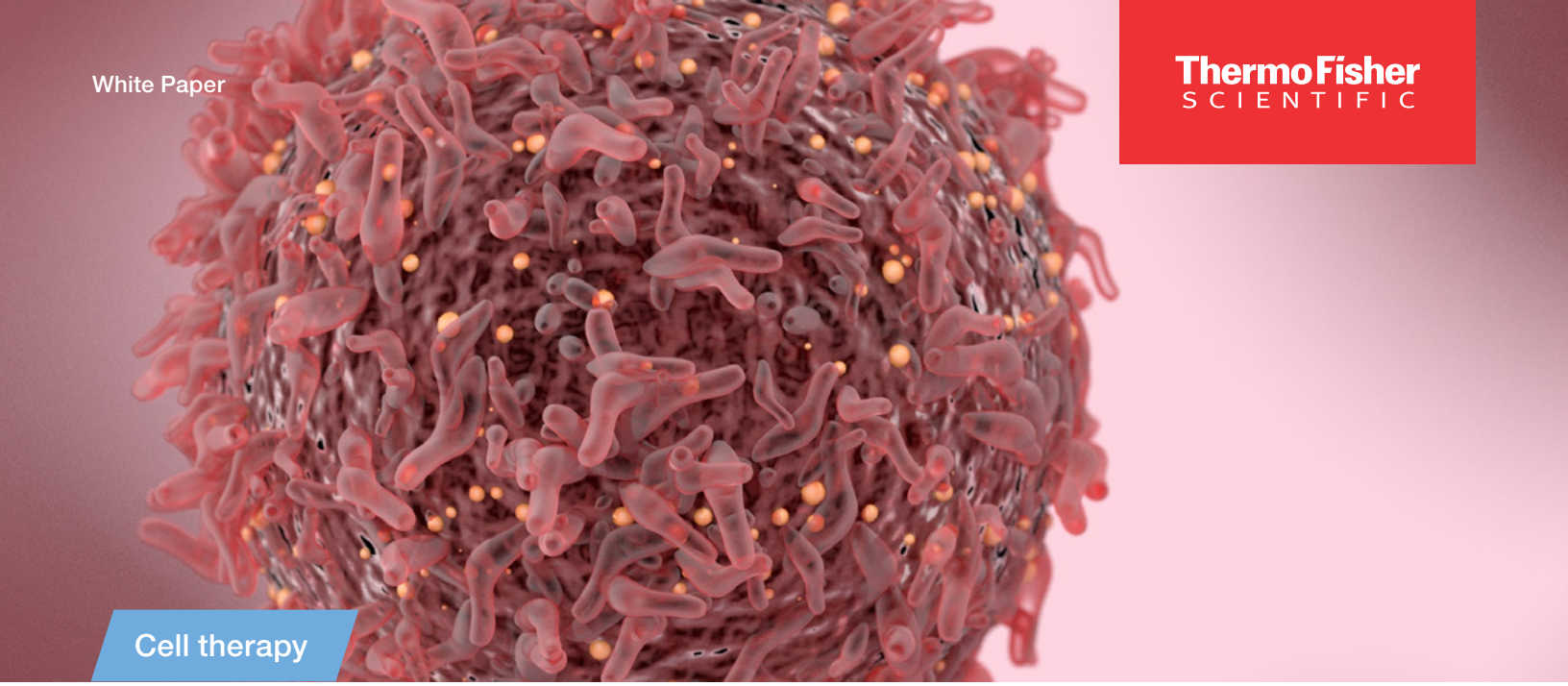
White Paper



Cell therapy

Accelerating cell therapy development: Investigating the impact of increasing pump speed on the Dynabead removal process

Keywords:

Cell therapy, accelerating, scale-up, Dynabead removal, CAR T, DynaCellect

The global cell therapy market is growing rapidly, and developers are eager to move quickly towards commercialization. Isolating and activating target T cells is a vital step in the production of these life-changing cell therapies. The process of isolation can use paramagnetic beads coated with antibodies to bind to receptors on the target cells, creating a magnetic complex. An instrument with magnetic capability can then isolate the T cells from the sample. Following this, the paramagnetic beads must then be removed prior to downstream processes to help prevent residual bead material in the final product. Failure to remove the beads could directly impact the safety of treatment for patients who are already vulnerable. Consequently, effective bead removal is vital to the production of safe, efficacious therapies.

Carrying out the bead removal process both thoroughly and quickly is essential. In time-sensitive cell therapy manufacturing, efficiency is crucial; every hour that can be shaved off the workflow can equate to shorter time to treatment and reduced cost to manufacturers and patients. Another consideration is the impact of manufacturing time on the efficacy of the therapeutic product. Accelerating manufacturing can help reduce the need for steps such as freezing and thawing, which can affect cell viability [1].

For cell therapy developers, accelerating their workflow is therefore crucial, and increasing throughput for bead removal is one way to maximize efficiency. However, it is important to understand how this may affect cell viability and recovery and, as a result, the final drug product.

The challenges of rapid bead removal

The bead removal process can be accelerated by increasing pump speed of the chosen bead removal platform. This in turn will increase the force of the fluid stream passing through the system so that beads are removed more quickly, therefore reducing overall manufacturing time. As mentioned above, reducing manufacturing time is beneficial to help reach patients faster, increasing the cells' efficacy, and helping reduce cost of the treatment. However, despite these benefits, there are three main risks associated with increasing speed:

1. High numbers of residual beads: Increasing speed could result in dislodged beads that end up in the final therapeutic product.
2. Decrease in cell viability: As a result of the faster fluid stream, cells may be exposed to higher shear stress, possibly causing a deleterious effect.
3. Cells lost in the system: Higher speeds could result in lower cell recovery. With a limited number of cells to work with, loss of these valuable cells is problematic.

When increasing pump speed, it is important that these potential risks are kept in mind.

Gibco™ CTS™ DynaCellect™ Magnetic Separation System

The Gibco CTS DynaCellect Magnetic Separation System, an advanced, automated, closed platform, was developed to optimize and accelerate cell isolation and Gibco™ CTS™ Dynabeads™ magnetic bead removal while also addressing the risks involved. The system and single-use kits can help customers isolate the right cells, minimize failures in manufacturing, and reduce contamination, while decreasing variability and enabling increased robustness and precision.

Manual Dynabead removal solutions are labor-intensive and introduce the potential for human error. These manual methods also struggle with delivering reproducible results, and ultimately the capacity for failure or delay is a constant risk. The CTS DynaCellect system automates the process, delivering >86% isolation efficiency of target cells with 96% purity when used with Gibco™ CTS™ Dynabeads™ CD3/CD28.

Below, we outline the effect of increasing pump speed—from the recommended 50 mL/min to 150 mL/min—on cell recovery, cell viability, residual beads, and total run-time at a scale of 400 million target cells.

Cell recovery

Using the ChemoMetec NucleoCounter NC-200, it was found that increasing pump speed, and therefore accelerating the Dynabead removal process, did not impact cell recovery.

Comparable cell recoveries were observed when using the CTS DynaCellect Magnetic Separation System with CTS Dynabeads CD3/CD28 at both speeds, with 50 mL/min yielding an average recovery of 86% and 150 mL/min bead averaging 90% recovery of live cells (Figure 1).

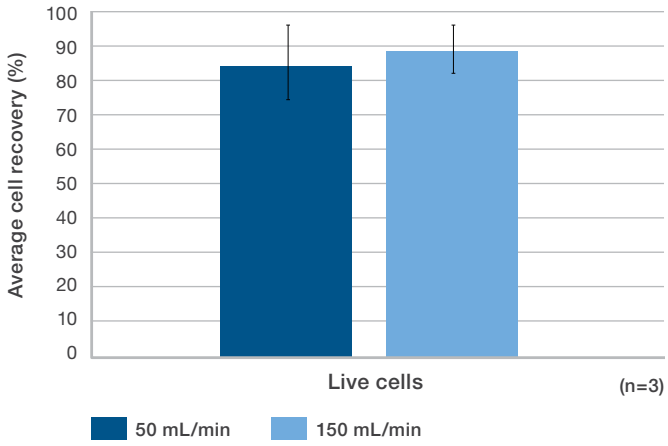


Figure 1. Cell recovery is comparable at both 50 mL/min and 150 mL/min speeds when using the CTS DynaCellect Magnetic Separation System.

Therefore, cell recovery was not compromised by an increase in speed and the higher speed was determined to be comparable to the recommended speed. This is because the CTS Dynabeads CD3/CD28 beads are effectively captured by the CTS DynaCellect magnet and retained in the bead removal bag. In addition, the peristaltic pump does not have detrimental shear effects on T cells at these speeds. It is therefore possible to increase pump speed up to 150 mL/min—and increase productivity—without impacting cell recovery.

Cell viability

Maintaining high cell viability is essential in the production of cell therapies as the cells are the drug. Achieving an adequate dose size (number of cells) is important, so any cells lost may impact the efficacy of the therapy. Alternatively, decreased cell viability can also result in a longer manufacturing process in order to achieve the number of viable cells needed.

Using the NucleoCounter, we found that increasing pump speed did not impact cell viability. Here, the 50 mL/min and 150 mL/min Dynabead removal speeds yielded comparable cell viabilities from input to output, with both speeds averaging approximately 80% cell viability (Figure 2).

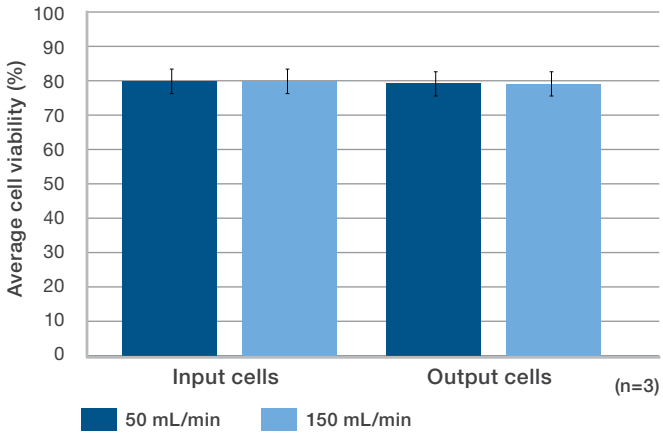


Figure 2. Cell viability is maintained at both 50 mL/min and 150 mL/min speeds when using the CTS DynaCellect Magnetic Separation System.

Residual Dynabeads

Residual CTS Dynabead CD3/CD28 numbers were observed in samples from three donors using visual counts with microscopy and hemocytometry. Residual Dynabead counts were similar at both 50 mL/min and 150 mL/min, and both were below the industry accepted criteria of 100 beads per $3x10^6$ cells (Figure 3).

Therefore, it is clear that increasing pump speed does not negatively impact the number of residual Dynabeads. As a result, it is possible to accelerate Dynabead removal up to 150 mL/min without risking the safety of the therapeutic product.

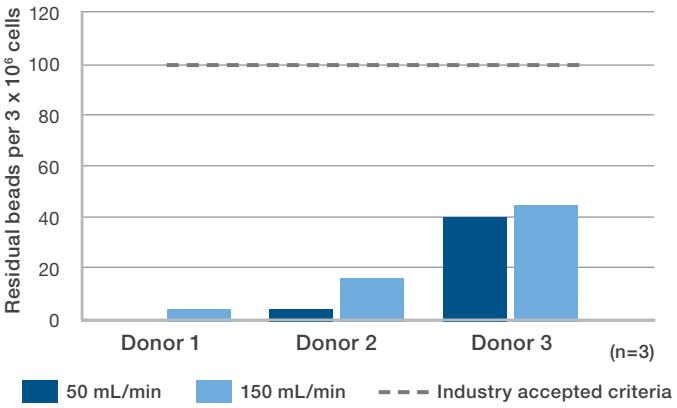


Figure 3. Residual bead levels were maintained well below industry acceptable criteria of 100 beads per $3x10^6$ cells at both 50 mL/min and 150 mL/min speeds using the CTS DynaCellect Magnetic Separation System.

Run time

As expected, we found that increased removal speeds yielded run-time savings when using the CTS DynaCellect system. The slower speed at 50 mL/min resulted in an average run time of 44 minutes, whereas the increased speed of 150 mL/min resulted in an average run time of 18 minutes, representing a 59% average run time reduction or average savings of 26 minutes per run (Figure 4).

The above results were obtained from runs using 400 million cells, isolated and activated at a 3:1 ratio, and it is important to note that scale may impact the time savings.

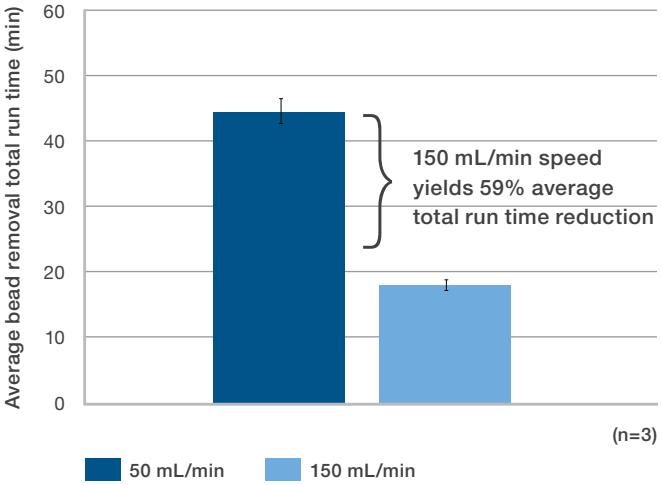


Figure 4. Total run-time was reduced by an average of 59% using the CTS DynaCellect Magnetic Separation System when pump speed was increased from 50 mL/min to 150 mL/min.

Conclusion

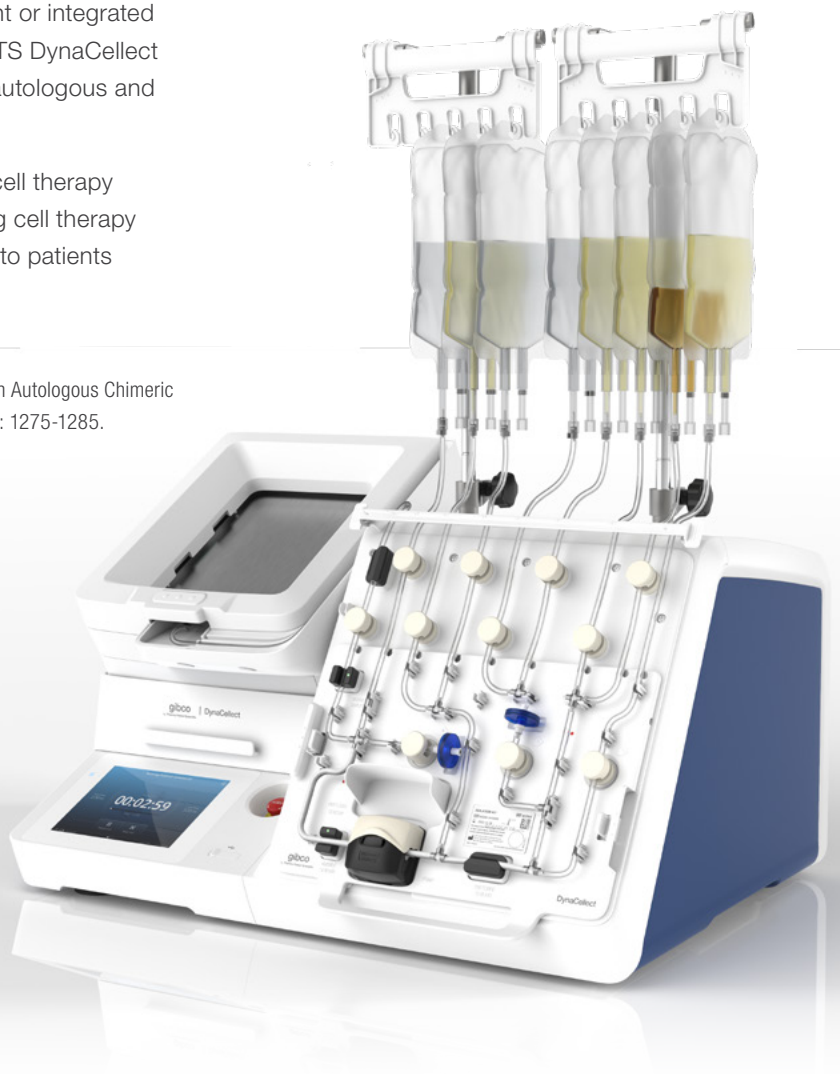
Based on our findings, the CTS DynaCellect Magnetic Separation System can accelerate Dynabead removal without negatively impacting cell recovery or viability. Furthermore, at increased speed, residual Dynabead levels were kept below industry accepted criteria.

Increasing pump speed therefore presents a promising way of accelerating the Dynabead removal step of the cell therapy production process without any negative impacts on output. This could ultimately help cell therapy developers get effective therapies to the patients who need them quickly and efficiently. Furthermore, by using a continuous flow process for Dynabead removal, the processing volume is potentially unlimited, unlocking doors for scaling up. As a standalone instrument or integrated digitally and physically into any workflow, the CTS DynaCellect system offers scalability and flexibility for both autologous and allogenic workflows.

Overall, the CTS DynaCellect system can help cell therapy manufacturers stay ahead in the rapidly growing cell therapy industry and fast-track their essential therapies to patients who need them most.

References

[1] Panch, Sandhya R. et al. (2019) Effect of Cryopreservation on Autologous Chimeric Antigen Receptor T Cell Characteristics. Molecular Therapy 27.7: 1275-1285.



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