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LIFE SCIENCES

More Cells, Better Science.

Effective Dead Cell Removal using Microbubbles Delivers Incredibly High Yield

Dead cell contamination is a common problem that has a negative impact on downstream applications like cell separation, cell culture, cell therapy, or single cell sequencing. Existing methods for dead cell removal (DCR) can be harsh on delicate cells of interest, yield too few viable cells, or have too high a cost or time investment to be practical. There is a strong need for fast, easy, and gentle processing to remove dead cells that can enable the use of samples that would otherwise be discarded, and Akadeum's next-generation Buoyancy Activated Cell Sorting (BACS™) microbubbles offer a revolutionary, buoyant approach that delivers more cells for better science.

Stop losing your precious samples to outdated processes! Akadeum's Dead Cell Removal Microbubbles are simple and effective.

Using a streamlined 3-step workflow (mix to bind, spin to separate, aspirate to remove) that's fast to perform and exceptionally gentle on delicate cells of interest, you are able to retain more of your precious cells to bring to downstream processes. Akadeum's Dead Cell Removal Microbubbles offer high viability as well as high yield.

KEY POINTS OF NOTE:

- **Akadeum's Dead Cell Removal Microbubbles are simple and effective.**
- **Streamlined workflow: Mix to Bind · Spin to Separate · Aspirate to Discard**
- **More cells, better science. Microbubbles deliver high yield & viability.**

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Introduction

Dead cells can have a negative impact on numerous downstream applications. For instance, dead cells and their released components can impact the activity and physiology of healthy neighboring cells, increase background noise in sequencing experiments leading to misinterpretation of collected data, and contribute to extended cell sorting times or lower overall sample purity. In general, dead cell contamination in the sample being processed ultimately contributes to inferior results.

Existing methods for dead cell removal (DCR) like flow sorting, low-speed centrifugation, density gradient separation, or magnetic bead-based removal of dead cells all have their own drawbacks and limitations. These techniques can be harsh on delicate cells of interest, yield too few viable cells, or have too high a cost or time investment to be practical. There exists a strong need for fast, easy, and gentle processing to remove dead cells that can enable processing of samples that would otherwise be discarded.

Akadeum's next-generation cell isolation technology using Buoyancy Activated Cell Sorting (BACS™) microbubbles is a revolutionary, buoyant approach that eliminates many of the existing technical hurdles that are limiting improvements or effectiveness of cell separation processes. Given that Akadeum's microbubble technology overcomes long-standing headaches in sample preparation, applying this technology to dead cell removal is an impactful way to leverage the microbubble removal workflow. Akadeum's targeted removal of dead cells is achieved through the selective capture of cells with exposed phosphatidylserine (PS) using Annexin V conjugated BACS™ microbubbles. Once mixed with the sample, the Annexin V BACS™ microbubbles capture dead cells and float them to the surface for removal, leaving the untouched cells of interest happy, healthy, and ready for downstream use.

Leveraging the Mechanics of Apoptosis to Capture Dead Cells

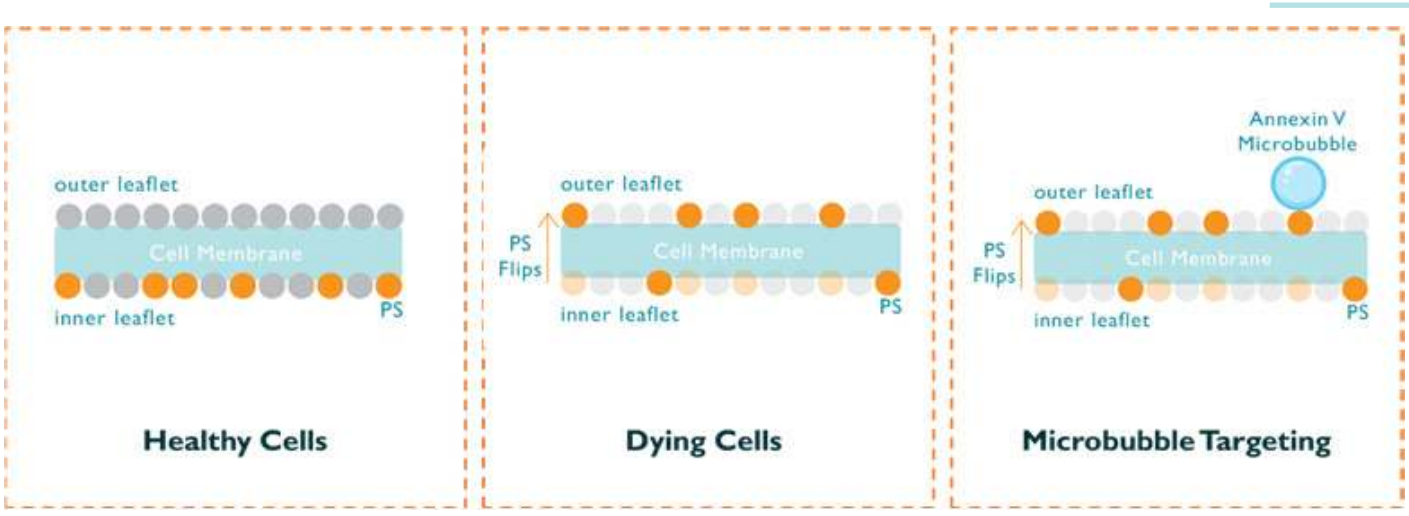


Figure 1: Mechanism of Action for Akadeum's Dead Cell Removal Microbubbles. *Diagram demonstrating the naturally occurring phospholipid asymmetry that is disrupted during apoptosis and how changes occurring at the cell membrane can be used for targeting dead and dying cells with Akadeum's Dead Cell Depletion Microbubbles.*

Apoptosis, the natural process of cellular death, is defined by a series of biochemical events that lead to characteristic changes in the cell. During apoptosis, phosphatidylserine (PS) – a phospholipid normally found on the inner surface of the plasma membrane – is redistributed to the extracellular surface 1-4. This relocation of PS to the outside of the cell membrane functions as a cellular marker of apoptosis, making it an effective target for identifying dead cells within a biological sample. The exposed PS is able to engage with a number of molecules, including Annexin V 5. It is this PS-Annexin V interaction that can create an effective mechanism by which dead cells can be removed (Figure 1).

A Faster and Easier Approach to Dead Cell Removal with Akadeum Microbubbles

Particle-based methods are among the most popular options for depleting unwanted cells from biological samples. Chief among those options are magnetic beads and buoyant microbubbles. A comparison of the two methods for dead cell removal highlights the additional handling, sample transfers, and extra steps associated with magnetic-based protocols (Figure 2A). The cumulative effect of these differences results in longer processing times observed with both column free and column-based magnetics (Figure 2B). Ultimately, the additional handling and processing time can negatively impact the user experience and quality of the purified sample.

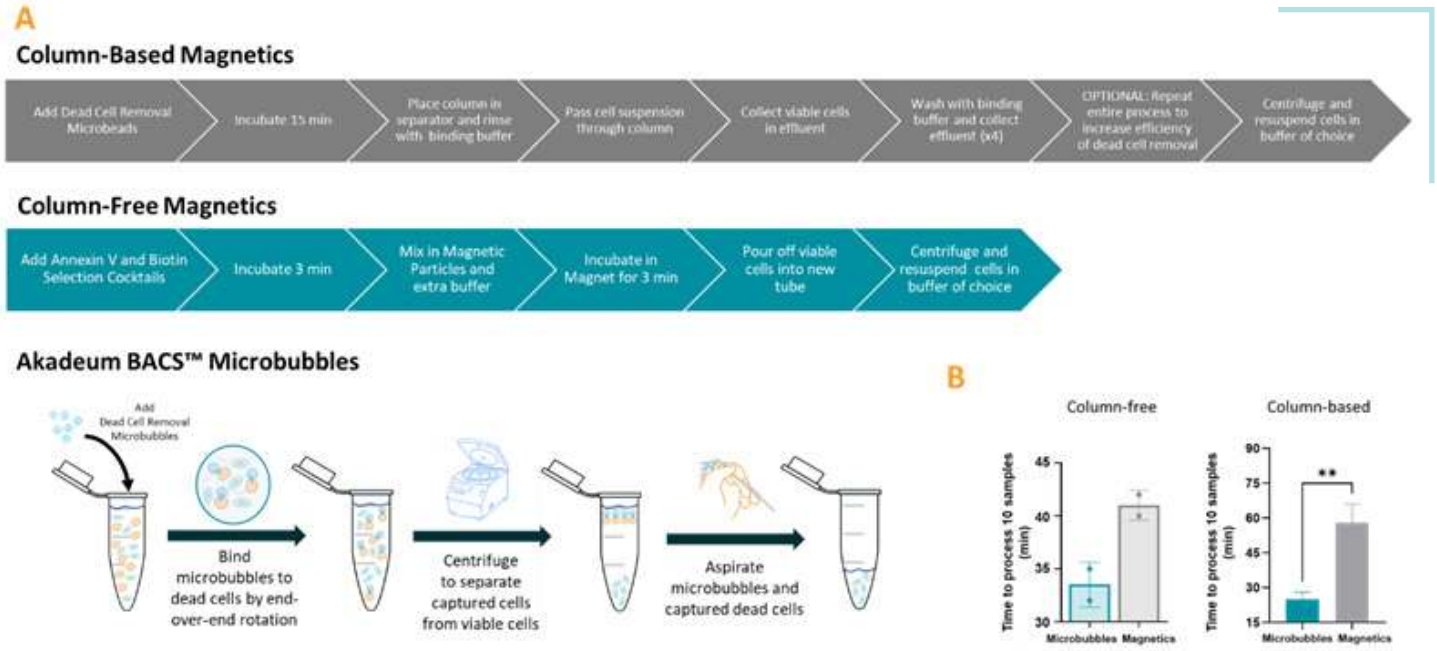


Figure 2: Akadeum's Simplified Workflow Cuts Steps and Saves Time Versus Magnetic Dead Cell Removal Protocols. *A) Example magnetic and microbubble dead cell removal workflows demonstrate Akadeum's simplified protocol. B) Bar graphs depicting the time required to process 10 samples at once with microbubbles or column-free magnetics (left) and the time required to process six samples at once with microbubbles or column-based magnetics (right) highlight the time saved using Akadeum's dead cell removal platform..*

With Akadeum's microbubble removal workflow, there is only a single pipetting step and one incubation step where the microbubbles will seek out and bind to the dead and dying cells. The separation of the microbubbles and captured cells happens at the same time as the untouched viable cells are pelleted, and in the final step, the microbubbles, captured cells, and supernatant are all removed in one simple, easy vacuum aspiration step. This fast and easy workflow saves time when compared to both column-free and column-based magnetic sample preparation.

Effectively Deplete Dead Cells from a Variety of Sample Types using Microbubbles

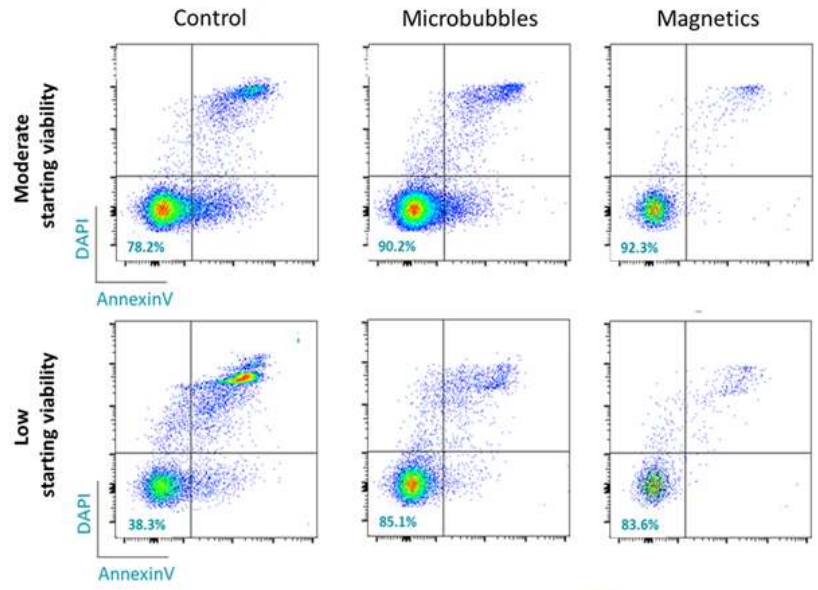
Akadeum's microbubble approach to dead cell removal is not only fast and easy, it is also effective.

To demonstrate effective depletion of dead cells, microbubble and magnetic dead cell removal protocols were used to deplete dead cells from mouse splenocyte (Figure 3A-B) and human PBMC (Figure 3C) samples that had been cultured at 4°C overnight to induce apoptosis.

After dead cells were depleted, the samples were stained with the viability dyes Annexin-V and DAPI (4,6-diamidino-2-phenylindole) and analyzed by flow cytometry.

Regardless of starting viability or sample type, Akadeum's Dead Cell Removal Microbubbles produced an enrichment of viable cells comparable to or better than the column-free or column-based magnetic protocols.

A



B

Moderate starting viability

Dead Cell Removal Method	Viable CD45+ Cells (%)
Control	77.9 ± 1.8
Microbubbles	91.1 ± 0.6
Column-free magnetics	92.7 ± 0.5

Low starting viability

Dead Cell Removal Method	Viable CD45+ Cells (%)
Control	37.2 ± 1.2
Microbubbles	85.0 ± 2.5
Column-free magnetics	83.2 ± 3.0

C

Low starting viability

Dead Cell Removal Method	Viable CD45+ Cells (%)
Control	39.9 ± 0.3
Microbubbles	74.8 ± 0.5
Column-based magnetics	65.1 ± 1.2

Figure 3: Dead Cells Are Effectively Removed with Microbubbles or Magnetics *A) Representative density plots depicting enrichment of viable mouse splenocytes from samples with low or moderate initial viability following microbubble or column-free magnetic depletion of dead cells. B) Tables summarizing enrichment of viable mouse splenocytes. C) Table summarizing enrichment of human PBMCs from samples with low initial viability after removal of dead cells with Akadeum's microbubbles or a column-based magnetic competitor.*

Quantity and Quality: Akadeum's Dead Cell Removal Microbubbles Provide Both

The microbubble platform is exceptionally gentle on viable cells of interest, which becomes especially apparent when looking at the number of viable cells that are retained throughout the dead cell removal process. Akadeum's Dead Cell Removal Microbubbles enable significantly higher recovery of viable cells than was observed in both column-based and column-free magnetic processing, both of which suffer from significant loss of viable cells. As depicted in Figure 4, when various tprocessing ypes samples of including human PBMCs (Figure 4A) and mouse splenocytes (Figure 4B) Akadeum's DCR microbubbles were able to achieve significantly higher recovery of viable CD45+ cells from the starting sample than was obtained using column-based magnetics (Figure 4A) or column-free magnetics (Figure 4B). Akadeum's Dead Cell Removal Microbubbles resulted in a 2-fold increase in recovery (>90% recovery vs. <50% recovery) when compared to column-based magnetics, and a 5-fold increase in recovery (>85% recovery vs. <20% recovery) when compared to column-free magnetic processing.

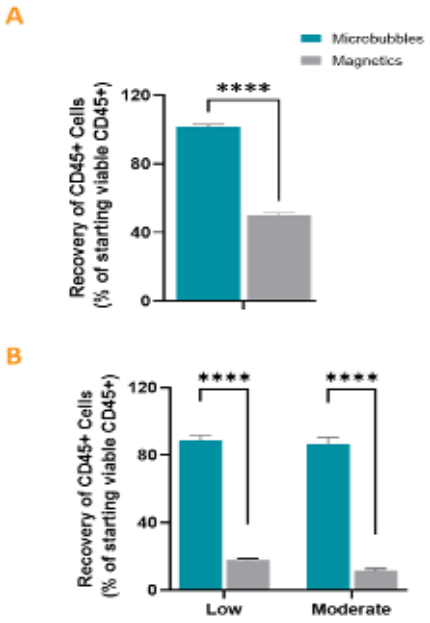


Figure 4: Significantly More Viable Cells Recovered Using Microbubbles *Bar graphs depicting the percentage of viable CD45+ PBMCs (A) or mouse splenocytes (B) from the starting sample that remained in the sample through the removal of dead cells using column-based magnetics (A) or column-free magnetics (B).*

Akadeum's Dead Cell Removal Microbubbles Successfully Lower Contaminating Ambient RNA from Single Cell Sequencing Preps

Decreasing ambient nucleic acids during dead cell removal processing could greatly improve resolution of single cell sequencing experiments (Figure 5).

To measure the impact of microbubble-based processing on ambient RNA, the supernatant was collected from Jurkat samples contaminated with dead cells both before and after the microbubble-based dead cell removal workflow being performed. RNA was then extracted from these supernatant samples and quantified by Qubit. As illustrated in Figure 5, samples with dead cells removed using Akadeum's microbubbles had greatly lowered ambient RNA contamination.

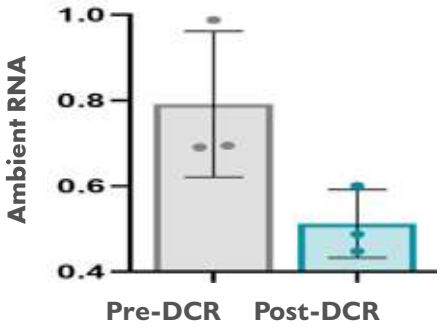


Figure 5: Akadeum's Dead Cell Depletion Eliminates Ambient RNA from Single Cell Sequencing Samples. *Bar graph of ambient RNA concentrations in supernatant from control and depleted samples demonstrates effective reduction in contamination.*

Conclusion

Sample preparation and analysis is frequently complicated by the presence of dead cells, which leads to negative downstream impacts like poor sort efficiencies, extended sort times, and overall reduction in purity. Dead cell contamination can also have negative effects on downstream applications like cell culture, cell therapy, or single cell sequencing. Overall, the presence of dead cells in the sample being processed ultimately produces inferior results. Traditional methods for dealing with dead cells have numerous drawbacks and limitations – like harsh processing conditions, insufficient recovery of viable cells, and high cost and time investments – that make them inadequate solutions.

To address the challenges presented by the presence of contaminating dead cells, Akadeum has developed a novel Buoyancy Activated Cell Sorting (BACS™) microbubble approach for depleting dead cells from biological samples. There exists a need for fast, easy, and gentle processing that can enable effective and efficient processing of samples that would otherwise be discarded, and Akadeum's next-generation microbubble approach eliminates many of the existing technical hurdles that are limiting improvements or effectiveness in dead cell removal workflows today.

Akadeum's targeted depletion of dead cells is achieved through the selective capture of cells with exposed phosphatidylserine (PS) using Annexin V conjugated BACS™ microbubbles. Once mixed with the sample, the Annexin V BACS™ microbubbles capture dead cells and float them to the surface for removal, leaving the untouched cells of interest happy, healthy, and ready for downstream use.

	Column-Free Magnetics	Column-Based Magnetics	Akadeum's Microbubbles	Key Takeaway
Improved Final Viability	✓	✓	✓	All three approaches **improve final viability**
Time to Process	Moderate	Slow	Fast	The microbubble workflow is **fast to perform**
Recovery (Range observed)	≤ 67.3%	≤ 50.1%	≥ 86.5	The microbubble workflow offers **exceptional recovery**

Figure 6: Summary Table. *Table summarizing the comparative performance of column-free magnetics, column-based magnetics, and microbubble-based dead cell removal. While all three methods were able to achieve similar results in depletion, the microbubble approach was fast to perform and resulted in exceptional recovery of target cells of interest.*

Akadeum's Dead Cell Removal Microbubbles effectively remove PS+ cells while providing significantly better recovery of target cells than magnetic bead-based DCR. The microbubble workflow is significantly faster than magnetic DCR processing, which can help to deliver healthier cells for downstream applications. Furthermore, Akadeum's DCR Microbubbles significantly reduce ambient RNA. Taken together, these results provide a firm foundation for the development of a microbubble solution to dead cell removal.

With Akadeum's Dead Cell Removal Microbubbles, effective dead cell removal is achieved using a fast and easy 3-step protocol (mix to bind, spin to separate, aspirate to discard) that enables downstream use with high yield and viability.

Partner with Akadeum:

Dead Cell Removal is only one of the most recent applications where Akadeum's microbubble approach to separation is disrupting the status quo. Akadeum's microbubble technology is the next generation in separation techniques, and its inherent flexibility allows for targeted isolation of any number of targets – everything from cells to nucleic acids to proteins or chemicals. Our elegant and easy-to-use approach enables faster, more accurate workflows with exceptionally gentle processing that increases throughput and allows for greater recovery of rare cells. Our microbubble platform is also uniquely scalable – in both the number of concurrent samples being processed as well as in fluidic volume. And key to all of this is our team that is committed to your success. We thrive on problem-solving and are dedicated to helping the researchers we work with see exceptional results. If you're interested in leveraging the microbubble platform to overcome existing hurdles in your processes, we want to hear from you! Get in touch with us today at info@akadeum.com to start a conversation.

References:

1. Chen W, et al. TGF-beta released by apoptotic T cells contributes to an immunosuppressive milieu. Immunity. 2001 Jun;14(6):715-25.
2. Gao Y, et al. Antiinflammatory effects of CD95 ligand (FasL)-induced apoptosis. J Exp Med. 1998 Sep 7;188(5):887-96.
3. University of South Australia. (2020, October 22). New approach to fighting cancer could reduce costs and side effects. ScienceDaily. Retrieved April 6, 2021 from www.sciencedaily.com/releases/2020/10/201022093956.htm
4. Ilicic, T., et al. Classification of low quality cells from single-cell RNA-seq data. Genome Biol 17, 29 (2016).
5. Yang, S., Corbett, S.E., Koga, Y. et al. Decontamination of ambient RNA in single-cell RNA-seq with DecontX. Genome Biol 21, 57 (2020).

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About Akadeum Life Sciences

Akadeum Life Sciences, Inc, is a private early stage life sciences company based in Ann Arbor, MI. Founded in 2014, Akadeum was established with the goal of advancing human health. Akadeum creates advanced isolation products and fundamentally changes the way that isolating chemical and biological targets is approached. Akadeum's goal is to enable entirely new assays and workflows by delivering a microbubble platform technology that can isolate any sample, any volume, anywhere.

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