

Technical Bulletin

Cell Culture Media Manufactured in the Air Classifier Mill is Chemically Equivalent and Performs Comparably to Media Manufactured in the Continuous Pin Mill and in Ball Mills

SAFC Biosciences can produce cGMP dry powder cell culture media (DPM) using any one of three different milling methods — the traditional and industry-standard ball milling, as well as two other milling technologies: pin milling and air classifier impact milling. Both the pin mill system (CM) and the air classifier mill system (ACM) are considered continuous systems wherein the raw materials of a given batch of media are continually fed into the mill. The milled material is then blended to assure homogeneity of the final product. Both systems are capable of producing DPM lots of up to 4000 kg, assuming the bulk density of the DPM is 0.57 kg/L or higher. The maximum lot size is determined by the amount of powder that can physically fit into the blender, and so is dependent on the bulk density of each specific DPM formulation. The pin mill system is located in Lenexa, KS, with the air classifier mill system residing in St. Louis, MO.

The pin mill is a high-speed-impact mill consisting of a feed inlet, a set of two disks with concentric rings of pins and a product outlet. The raw materials are fed into the mill and

enter the center of the disk where they are centrifugally propelled outward and reduced in size by the rotating intermeshing pins.

The air classifier mill is also considered a high-speed-impact mill. Bulk material is fed into the grinding chamber where it is pulverized by impactors fixed to a high-speed rotating disk. This product is then drawn by airflow to an independently driven radial flow separator (air classifier wheel), which allows particles of the desired size to pass through while returning oversized particles back to the grinding chamber for further size reduction.

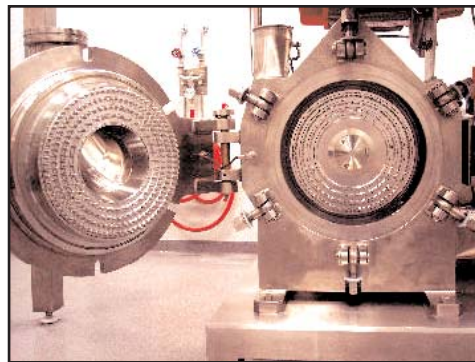


Figure 1. Pin mill chamber in the open position to allow for viewing of the disks.

The following analytical and cell culture data compare three lots of EX-CELL™ CD CHO Serum-Free Medium produced using each milling method and demonstrate that, despite the difference in milling technology, they are chemically equivalent and show no differences in performance. This media was chosen because it is complex, yet chemically defined (contains no hydrolysates) and serum-free.

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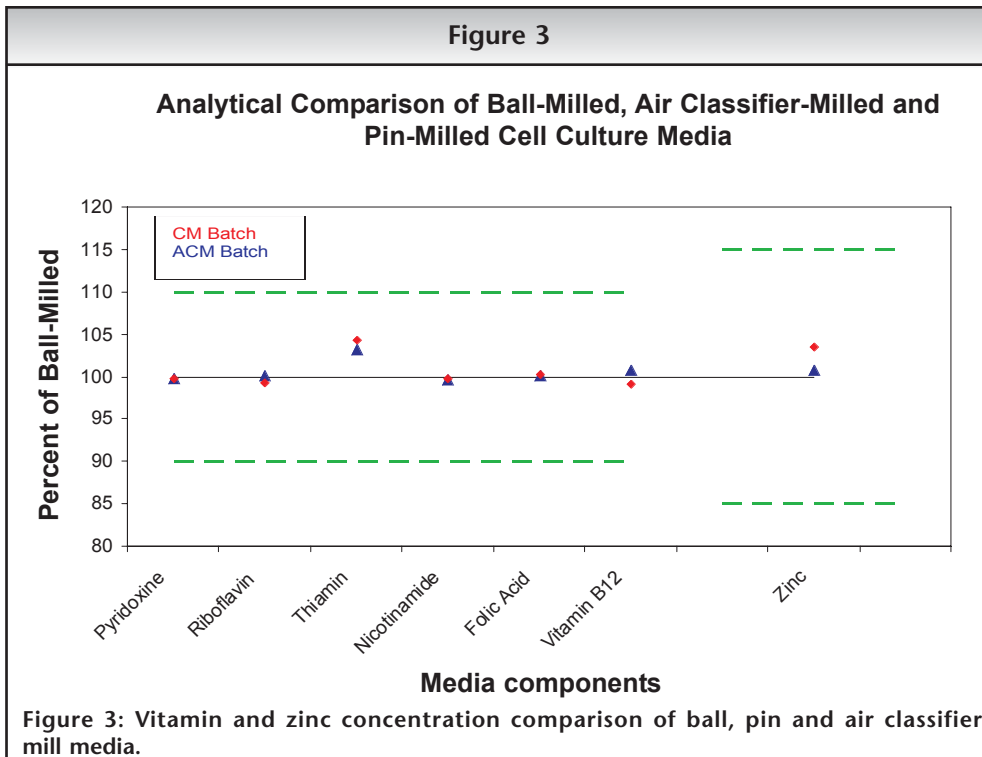
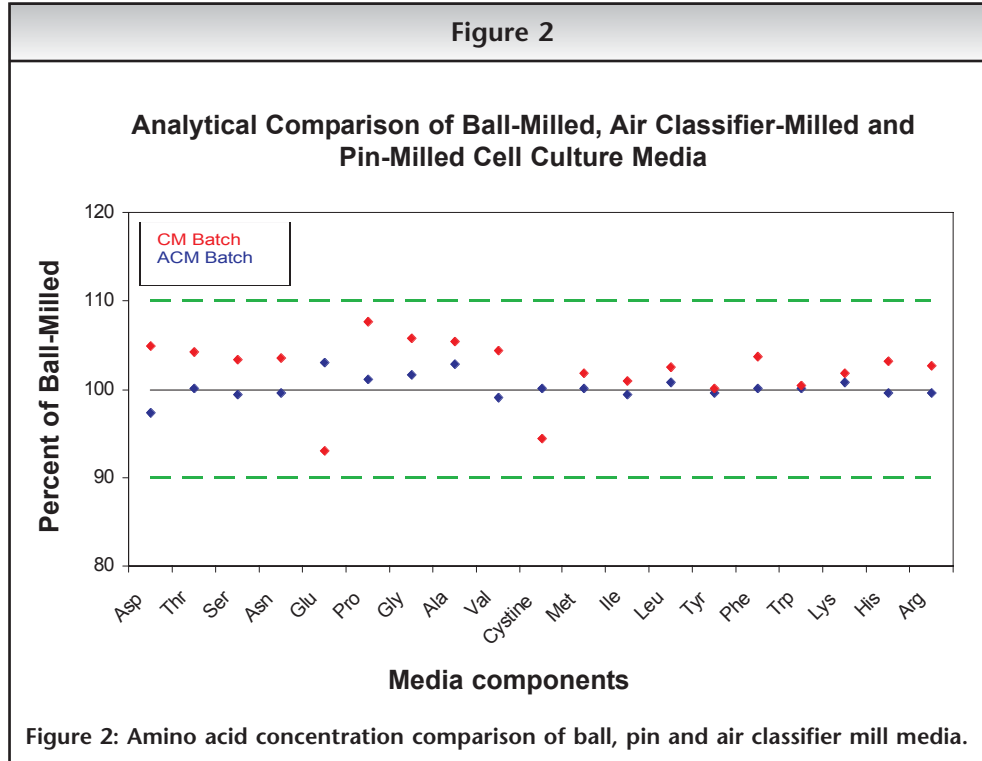
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Analytical Comparison

A lot of EX-CELL™ CD CHO ball-milled DPM was tested quantitatively for amino acids, vitamins and zinc, a trace metal, along with a beginning, middle and end sample of lots produced in the CM and ACM systems. Figures 2 and 3 show the results of that comparison.

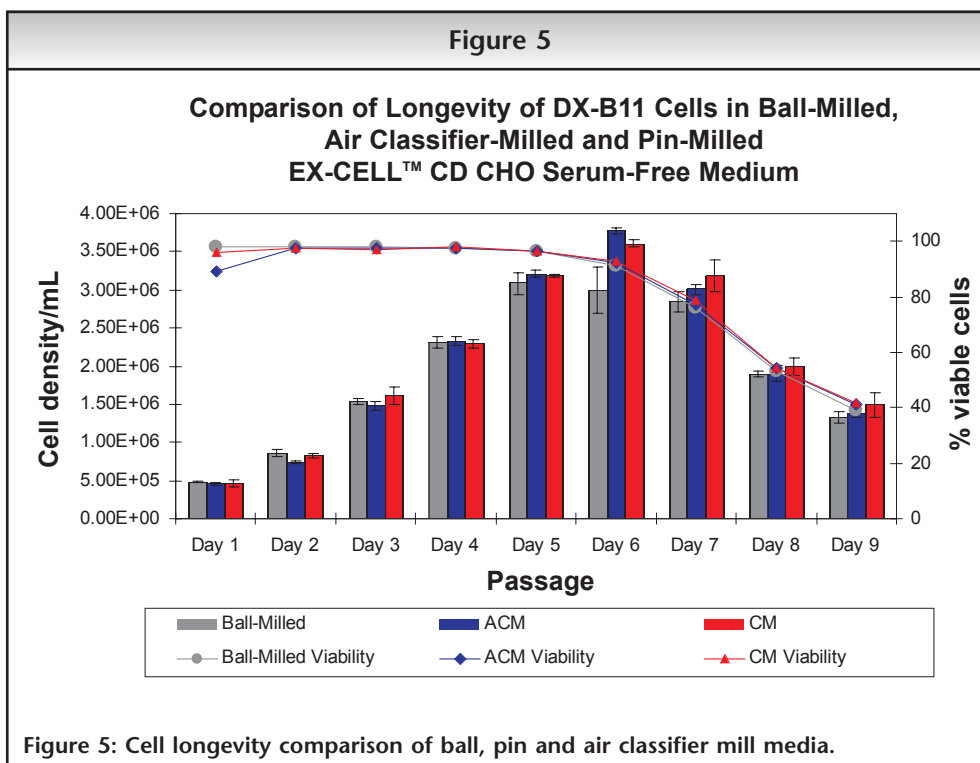
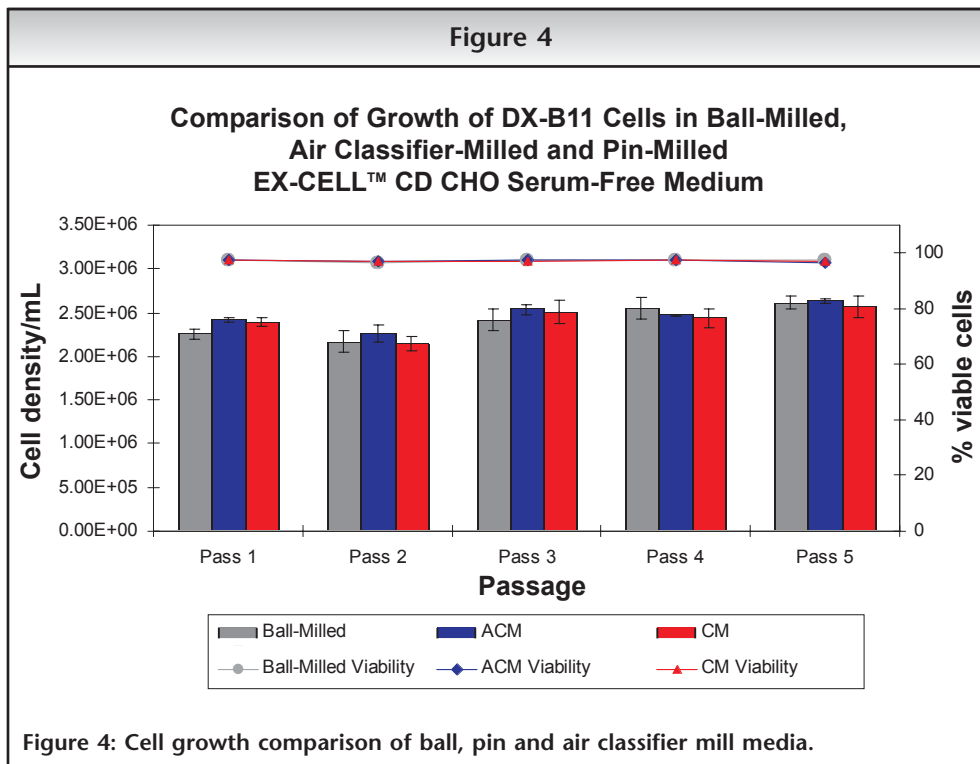
The normal assay variability range for testing components at these levels was determined to be 10% for the amino acids and vitamins and 15% for zinc based on preliminary studies and information from the testing labs. These data show the same medium formulation will contain the same amounts of individual components, within the normal assay variability range, regardless of being produced via ball mill, pin mill or air classifier mill.

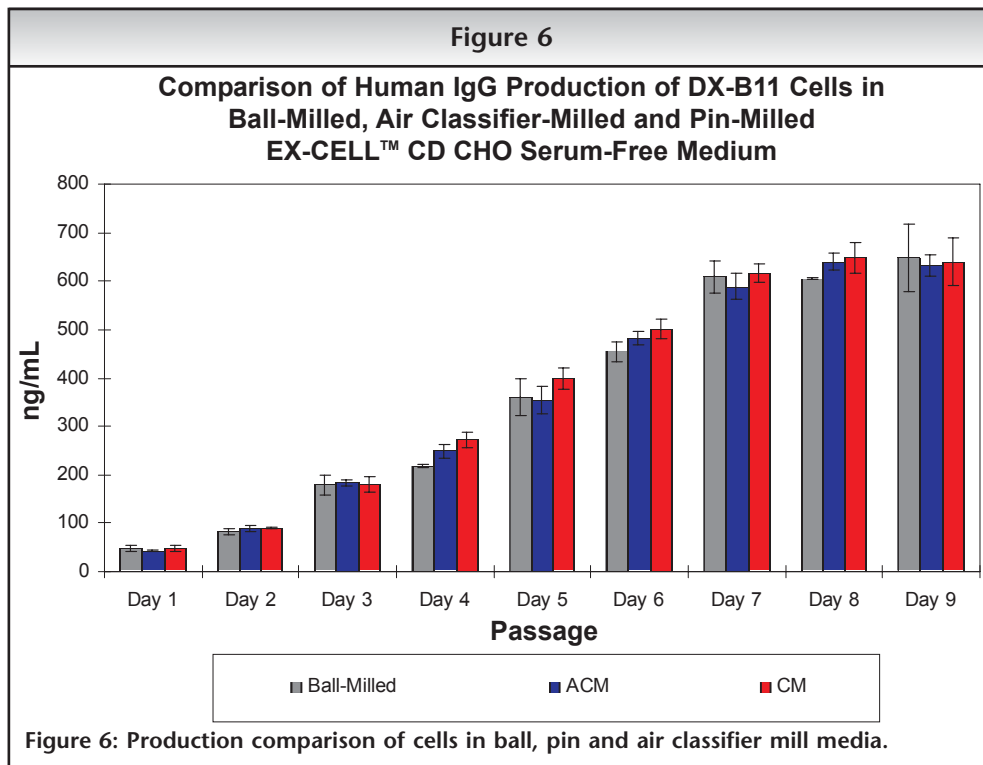


Biological Comparison

Another comparison of ball-milled, pin-milled and air classifier-milled media was performed using cell culture studies with the CHO (Chinese Hamster Ovary) cell line B13-24, a derivative of the DX-B11 line which secretes humanized immunoglobulin IgG. The studies evaluated cell growth over multiple passages, culture longevity and

antibody production in EX-CELL™ CD CHO. Typically, for cell studies, differences of up to 20% between groups is considered acceptable variability. It is illustrated in Figures 4, 5 and 6 that there are no significant differences in growth, viability or IgG production between the media.





No significant differences are observed in the performance of the media regardless of being produced via ball milling, pin milling or air classifier milling.

Figure 7

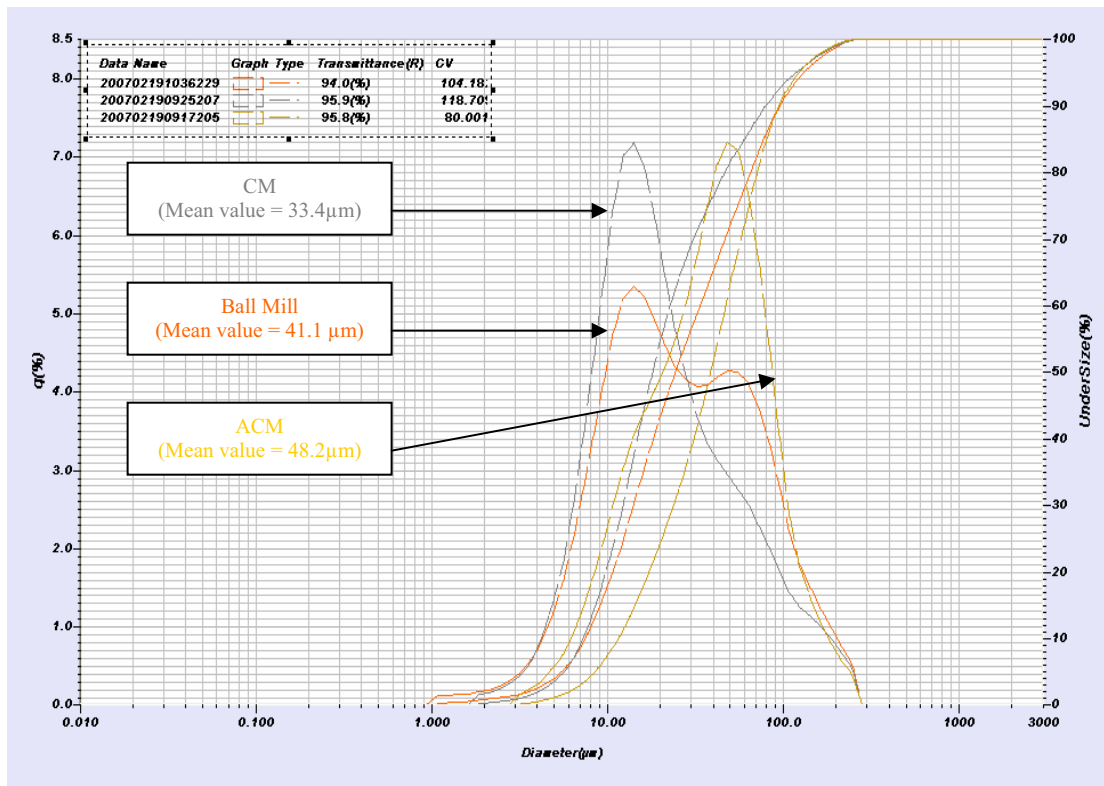


Figure 7: Particle size distribution curves of ball, pin and air classifier mill media.

Particle Size Comparison

The particle size distribution of EX-CELL™ CD CHO media produced in both the CM and ACM as well as in a ball mill was compared for informational purposes and is shown in Figure 7.

The "S"-shaped part of the curve represents the percentage of particles that are under a certain size. For example, 90% of the particles are $\leq 94.8 \mu\text{m}$, $\leq 79.7 \mu\text{m}$ and $\leq 96.1 \mu\text{m}$ for the ACM, CM and ball-milled media, respectively. Although the curve shape for the differently milled media are not identical, the mean particle size values of all three media are relatively similar. Any differences in particle size distribution that may exist did not affect other finished product testing such as solubility, pH or osmolality, and did not result in any difference in the performance of the medium with cells.

Summary

Both pin milling and air classifier milling can provide an alternative manufacturing process for customers considering risk management and contingency plans pertaining to their raw materials supply chain. Through both analytical and cell-based studies, it is demonstrated that continuous pin milling and air classifier milling produce quality media that is equivalent to ball-milled media.

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