

Technical Bulletin

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

In 2005 SAFC Biosciences opened a fully validated, state-of-the-art, cGMP-compliant continuous pin mill facility for manufacturing dry powder cell culture media (DPM) in Lenexa, Kan. The facility employs two different sized continuous pin mill systems, each consisting of a stainless steel pin mill (Hosokawa) combined with three-dimensional conical blenders to produce pharmaceutical-quality DPM in lots sizes of 10 to 4000 kg. The large-scale continuous pin mill system is referred to as the CM and the smaller-scale continuous pin mill system as the SSPM.

Pin mills are a type of high-speed-impact mills 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 raw materials are mixed in a blender both before entering and after leaving the pin mill chamber to ensure a consistent feed into the mill and a homogenous final product after being milled.

As part of the validation and qualification of the continuous pin mill, it was necessary to demonstrate that the DPM produced was both homogenous and comparable to the tradition and industry-standard ball-milled DPM. This was done by chemical analysis and cell growth studies comparing different lots sizes of pin-milled DPM against ball-milled control DPM.

Homogeneity

The pin-mill manufacturing process was fully validated to show that it uniformly blends components to achieve a homogenous and quality product. Testing proved that any given component was present at the same concentration at any given location throughout a batch of media. Multiple samples (approximately 10 - 40 depending on the lot size) throughout the lot were pulled, hydrated and sent for quantitative analysis of amino acids, vitamins and zinc. The media contained a very low level — approximately 20 ppm — of the trace metal zinc.

Percent relative standard deviation (% RSD) was chosen as the metric to evaluate homogeneity. The % RSD is the standard deviation divided by the mean and multiplied by 100 in order to be expressed as a percentage. It is used to indicate the variation between samples in a given population. The smaller the % RSD, the less variation and, in this case, the more homogenous the product. There will be an inherent amount of variability due to sample preparation and the analytical method itself, which will be larger for components present in smaller quantities.

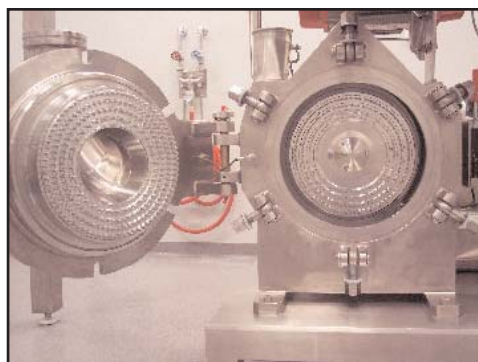


Figure 1. Pin mill chamber in the open position to allow for viewing of the disks.

Based on preliminary work and information from the testing laboratories regarding the assay variability, an acceptance criterion of $\leq 10\%$ RSD for the amino acids and vitamins and $\leq 15\%$ RSD for the trace metal zinc was chosen to indicate a homogeneous product.

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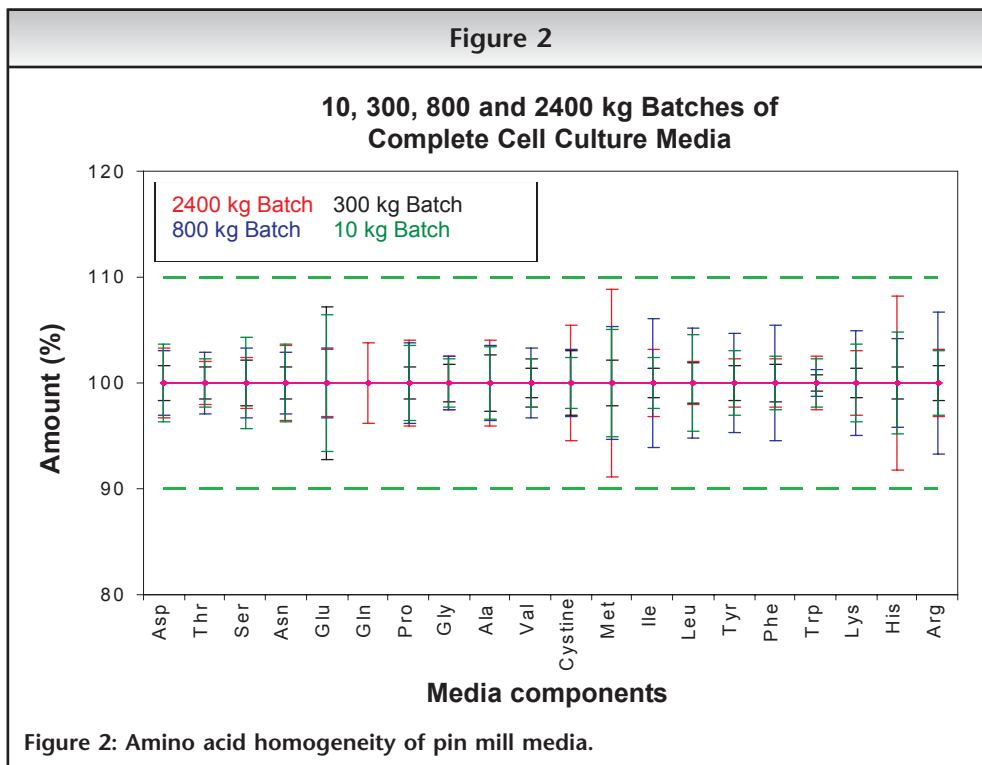
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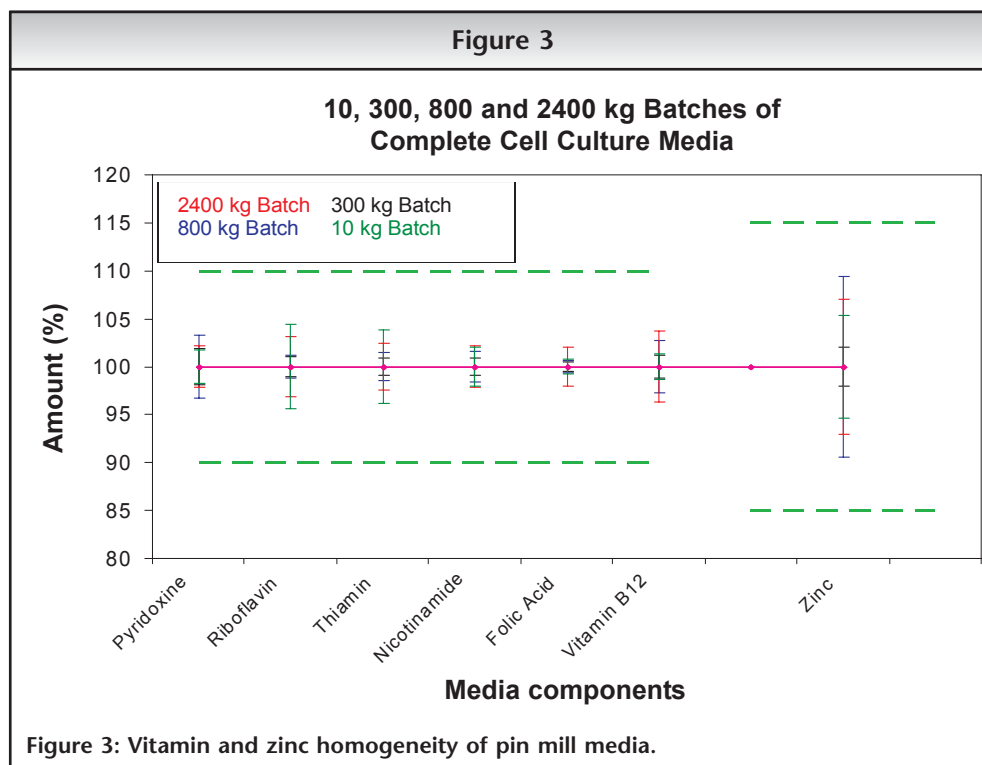
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Figures 2 and 3 show homogeneity results obtained as part of the validation for a classical medium (DMEM/F12 at 2400 kg batch size), a hydrolysate-containing serum-free medium (EX-CELL™ 302 Serum-Free Medium for CHO Cells at 800 kg batch size) and a chemically defined serum-free medium

(EX-CELL™ CD CHO Serum-Free Medium) at 300 and 10 kg batch sizes. All of the components met their criteria for being homogeneously distributed throughout the pin mill media regardless of batch size or formulation.



Note: Gln was not in the media formulations for the 300, 800 and 10 kg batches.

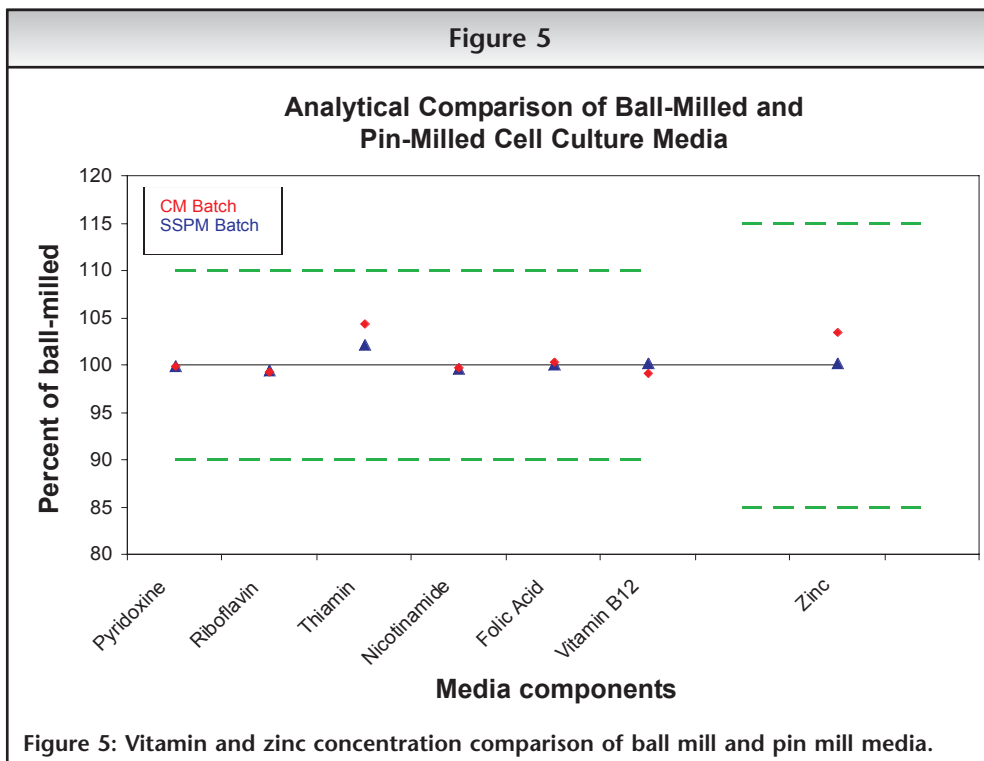
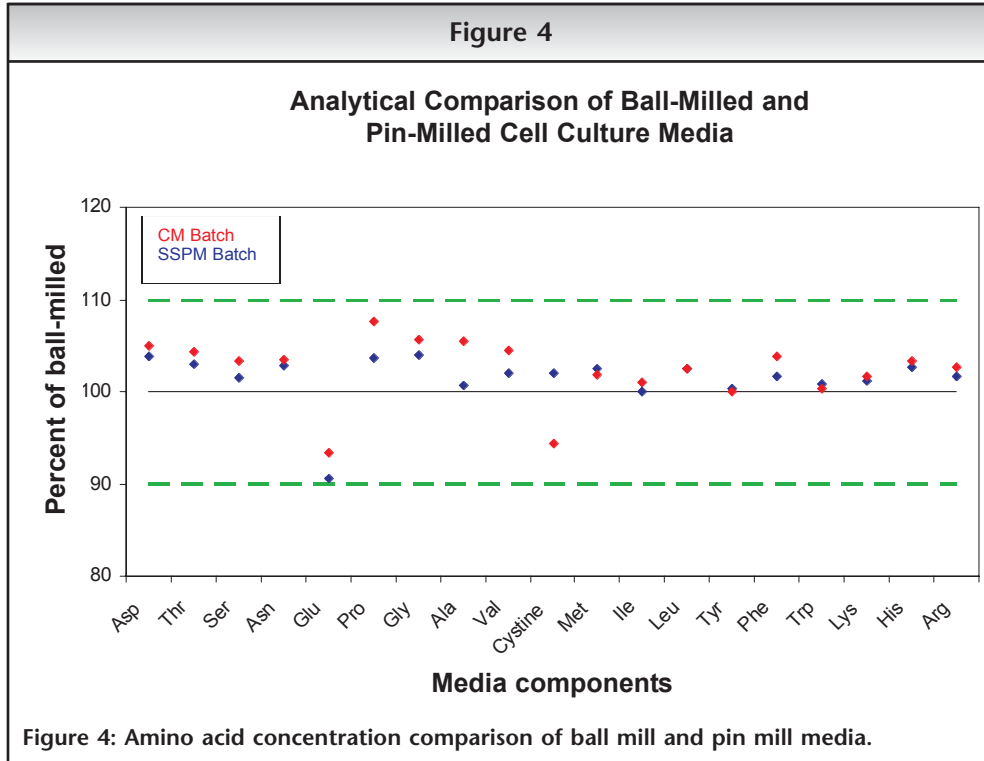


Analytical Comparison

An analytical comparison of pin-milled and ball-milled media was performed using EX-CELL™ CD CHO DPM. This medium was chosen because it is complex, yet chemically defined (contains no hydrolysates) and serum-free. One lot of ball-milled DPM was tested quantitatively for amino acids, vitamins and zinc, along with a beginning, middle and end

sample of pin-milled media made in both the CM and SSPM. Figures 4 and 5 show the results of that comparison.

These data show the same formulation will contain equal amounts of individual components, within the normal assay variability range, regardless of being produced via ball mill, large-scale (CM) or small-scale pin mill (SSPM).



Biological Comparison

A cell culture comparison of ball-milled and pin-milled media was performed with the CHO 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 media produced via ball milling and pin milling. Figures 6, 7 and 8 demonstrate that there are no differences in growth, viability or IgG production between the media.

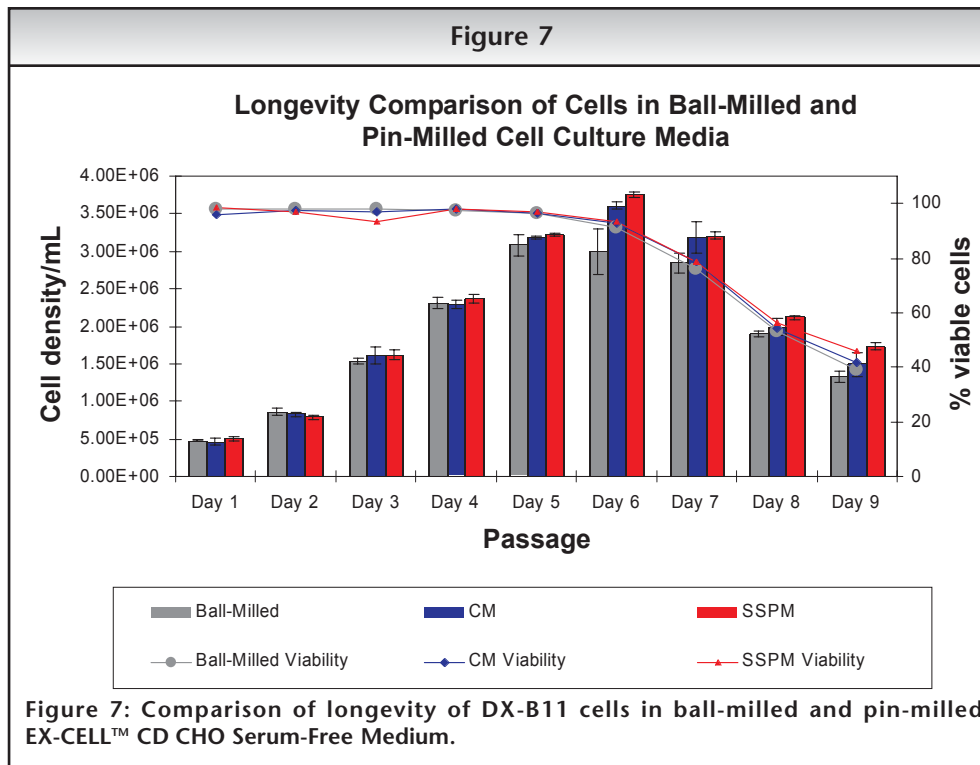
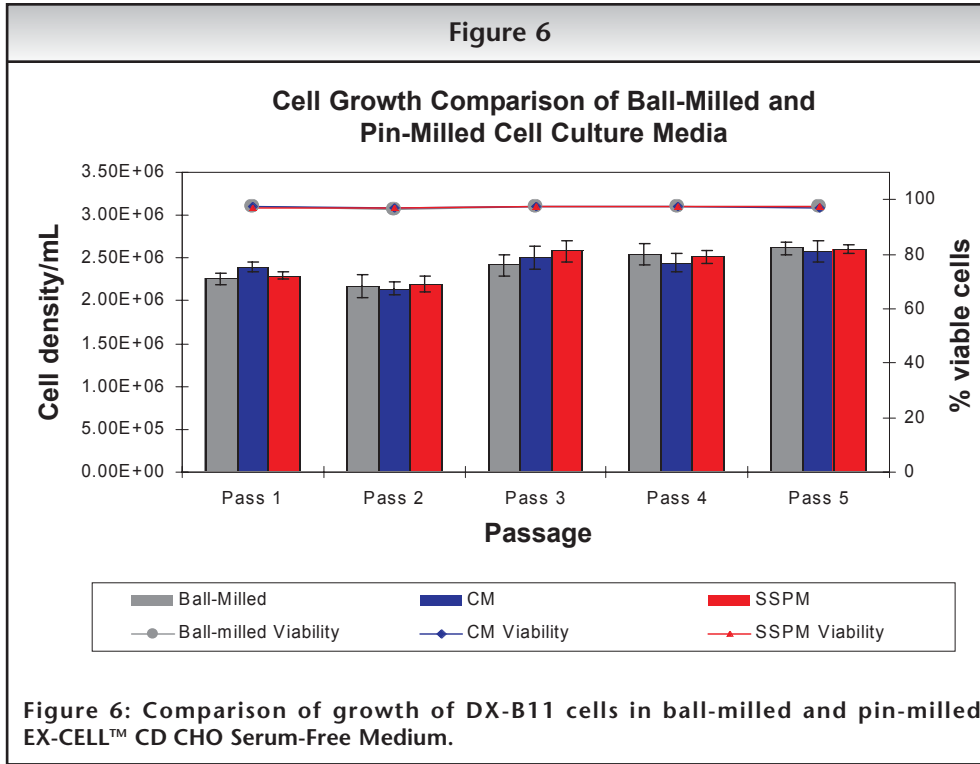
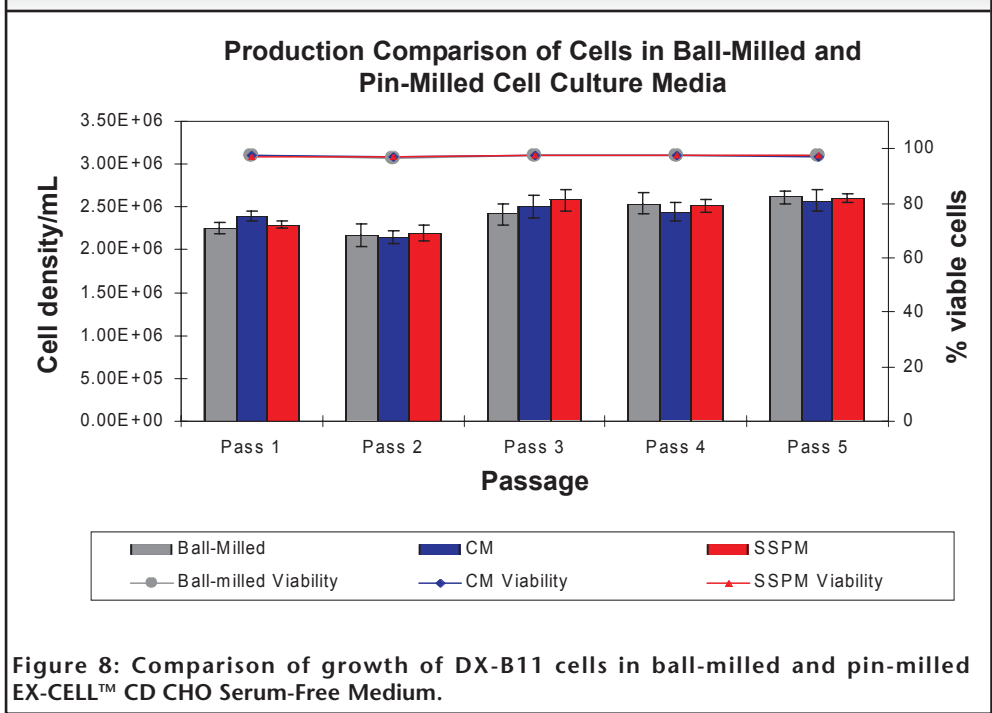


Figure 8

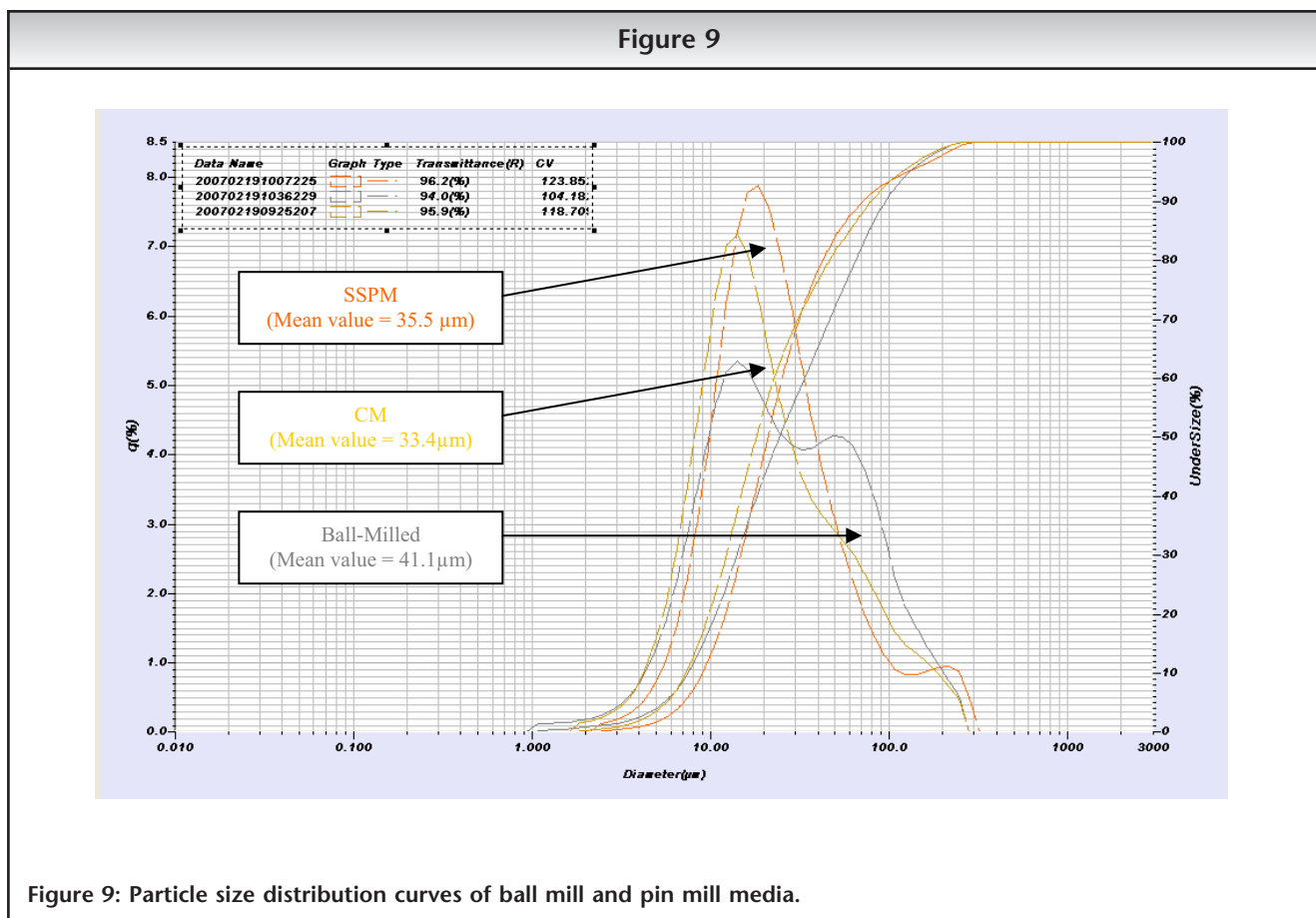


Particle Size Comparison

The particle size distribution of EX-CELL™ CD CHO media produced in the CM, SSPM and in a ball mill was compared for informational purposes and is shown in Figure 9.

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 $\leq 71.6 \mu\text{m}$, $\leq 79.7 \mu\text{m}$ and $\leq 96.1 \mu\text{m}$ for the SSPM, CM and ball-milled media, respectively. Whatever

differences in particle size distribution do exist, does not affect other finished product testing such as solubility, pH or osmolality, and does not result in any difference in the performance of the media on cells.



Summary

Through both analytical and cell-based studies it is demonstrated that continuous pin milling is an effective and scalable technique that can produce quality, homogenous cell culture media which performs equivalently to ball-milled media. Pin milling can provide an alternative manufacturing site and process for customers considering risk management and contingency plans pertaining to their raw materials supply chain.

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