# Significance of Cryogenic Broad Ion Beam Milling in Evaluating Microstructures of PLGA-based Drug Products

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## BACKGROUND

Poly Lactic-co-Glycolic Acid (PLGA)-based microspheres are being used as a controlled-release strategy for delivering a variety of active pharmaceutical ingredients (APIs) because of its good biocompatibility and biodegradability. Currently, there are no approved generic drug products that reference a PLGA-based brand-name (Reference Listed Drug, RLD) product [1]. This is attributed to the challenging development of PLGA-based generic drug products since subtle differences during manufacturing steps may result in significant changes in physicochemical properties, and subsequently affect the safety, stability and release characteristics of the drug product [2, 3]. Therefore, studies to characterize microstructural equivalence (Q3) among the PLGA-based drug products are of critical importance. The aim of this work is to characterize the internal microstructure and composition of PLGA-based microspheres. Towards that end, a robust method (Scanning Electron Microscopy (SEM) and ion beam milling) has been developed to assess the native morphology and drug distribution within these microspheres.

## MATERIALS AND METHODS

In this work, a cryogenic broad ion beam (cryo-BIB) milling system (TiC-3X, Leica) was utilized for sectioning PLGA-based microspheres (i.e., ARESTIN®, minocycline hydrochloride microsphere). The BIB milling system assisted with liquid nitrogen ($LN_2$) cooling was used to prepare cross-sectional samples. PLGA-based microsphere samples were prepared at 2kV acceleration voltage and 10 hours of milling time at -160°C.

The microsphere cross-sections were gold coated prior to imaging. Imaging and elemental analysis of samples were conducted using a field emission scanning electron microscope (FE-SEM, TESCAN Mira3) equipped with energy dispersive X-ray spectrometer (EDS, Oxford Max-80 SDD).

## RESULTS

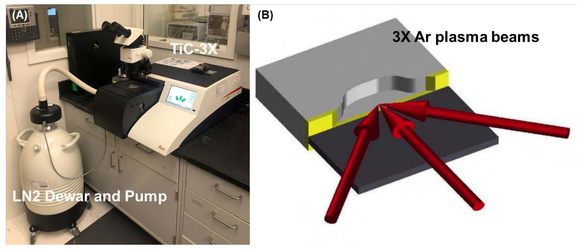
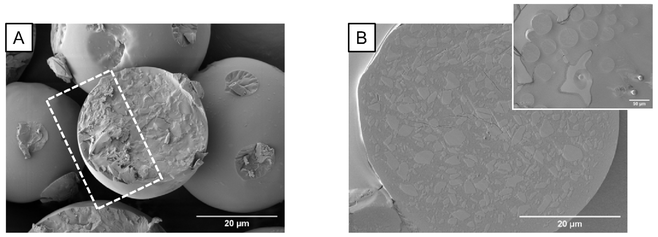



**Figure 1.** (A) The Leica TiC-3X Broad ion beam (BIB) milling system with cryo-stage at DPQR/OTR; (B) the BIB system has three Ar plasma beams to bombard the sample to create a smooth cross-sectional surface.

**Figure 2.** SEM micrographs of PLGA microspheres (ARESTIN®). (A) Blade cutting of microspheres show significant smear (white dashed box) and uneven cross-section. (B) SEM micrograph of a single microsphere processed using the broad ion beam milling reveal microstructures with no damage. The inset shows group of sectioned microspheres at lower magnification.



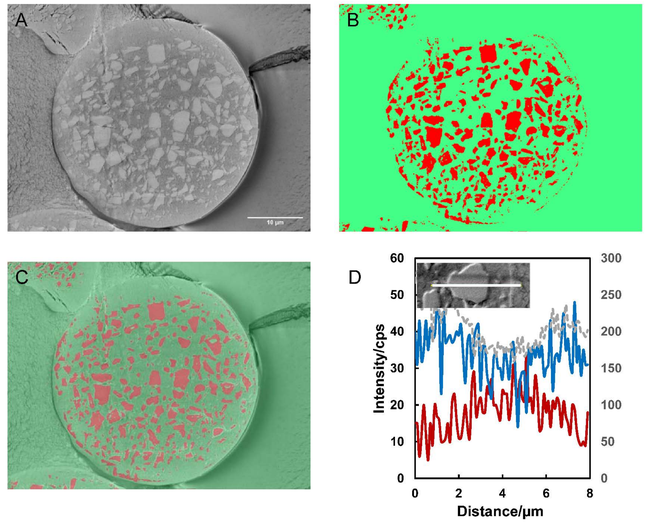




**Figure 3.** SEM micrograph showing drug distribution within the PLGA microspheres (ARESTIN®) with EDS analysis. (A) The gray scale micrograph reveals API particles (bright region) embedded within the PLGA matrix. (B) Post-processed binary image using ImageJ presents API particles in 'Red' with a green background. (C) Overlay of A and B micrographs. (D) API particles analyzed by EDS to determine the composition, including Carbon (Gray), Oxygen (Blue) and Nitrogen (Red).

**Table 1.** API particle size distribution showing 90% of API particle population smaller than ~3.8 µm based on area-weighted distribution.

| Unit (µm) | **Area-Weighted Distribution** |
|---|---|
| **$D_{10}$** | 0.9 |
| **$D_{50}$** | 2.1 |
| **$D_{90}$** | 3.8 |

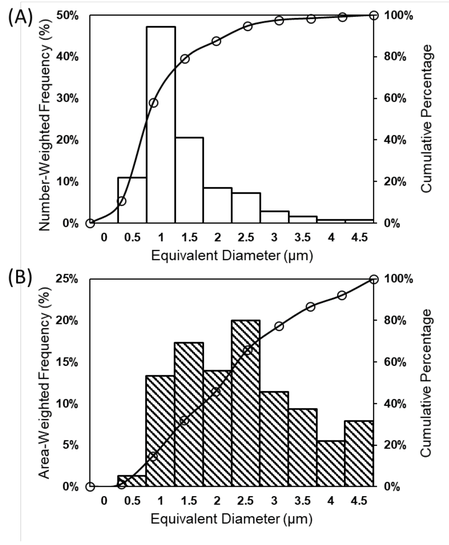



**Figure 4.** Summary of quantitative analysis of API particle size using number-weighted and area-weighted distribution. (A) number-weighted frequency (%) histogram with cumulative percentage. (B) Area-weighted frequency (%) histogram with cumulative percentage.

## CONCLUSIONS

- Cryo-BIB milling facilitated microstructural imaging and analysis of delicate samples like PLGA microspheres by preserving their native morphology with minimal damage during cross-sectional cutting compared to conventional methods.
- The high-quality cross-sections prepared with cryo-BIB technique revealed the microstructures inside the PLGA microspheres, which further enabled analysis of morphology, texture and composition of API distribution within the PLGA microsphere matrix using SEM-EDS.
- The cryo-BIB milling system combined with advanced microscopy provides an effective method for analyzing API particle size distribution based on image analysis. The cross-section surface prepared by this technique allowed precise evaluation of API particle size using area-weighted distribution.
- The BIB method developed in this study may be used as a prospective tool to investigate the equivalence of microstructural properties between the RLD and generic drug products. The research outcome will allow better understanding of these complex drug products and facilitate demonstration of microstructural equivalence.

## REFERENCES

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