PLGA Microsphere Formulation Development Guided by Microstructure Equivalence Assessment Using FIB-SEM Imaging and AI Image Analytics

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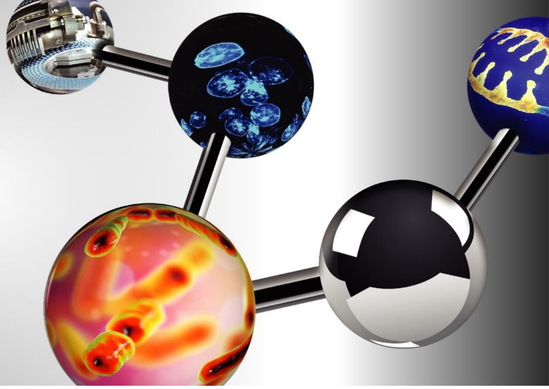

PURPOSE

Poly(lactic-co-glycolic acid) (PLGA) encapsulation continues to play a dominant role in the formulation of controlled release microspheres. As manufacturing process can affect the microsphere microstructure, qualitative (Q1) and quantitative (Q2) equivalence of PLGA products on their own may not be sufficient to ensure therapeutic performance equivalence. The assessment of microstructure equivalence, in terms of drug uniformity intra-sphere and inter-sphere, drug particle distribution, and porosity, is therefore critical to an effective characterization to support generic drug development and potential regulatory assessment of product quality and equivalence. Employing innovative microstructure imaging and quantification tools, this project demonstrates PLGA microsphere formulation development guided by microstructure equivalence assessment.

METHODS

- The in-house PLGA microspheres were prepared using a coacervation method. Briefly, the micronized minocycline hydrochloride powder was suspended in PLGA solution. Coacervation was induced by the addition of silicone oil to obtain coacervate droplets. The coacervation dispersion was then discharged into hexane under stirring to solidify the microspheres. Three in-house samples labelled D1153, D1228, and D1271 were prepared for comparison with the reference listed drug (RLD) Arestin® (D830).
- Focused ion beam-scanning electron microscopy (FIB-SEM), a thin sectioning imaging technique was used to analyze the four microsphere samples. A schematic example is shown in **Figure 1**.

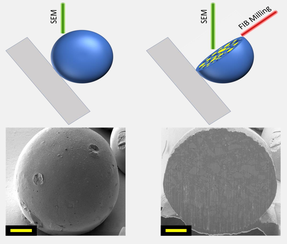

Figure. 1. Schematic representation of FIB-SEM imaging and representative SEM images.

- *In vitro* release tests of the four samples were conducted using a sample-and-separate method at 37℃ in 10 mM PBS (pH 7.4) containing 0.02% (v/v) Tween 20.

RESULTS

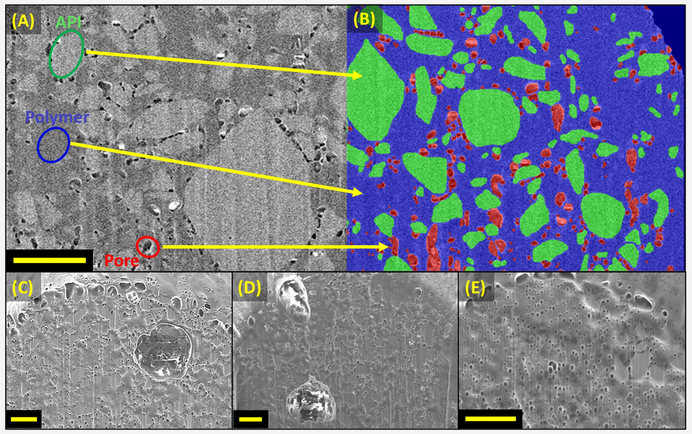

Figure 2. (A) FIB-SEM cross-sectional image of D830 (RLD) with polymer, API, and porosity identified. (B) Corresponding AI segmentation of D830 with phases indicated (polymer = blue, API = green, pores = red). FIB-SEM cross-sectional images with indicated phases for three in-house samples (C) D1153, (D) D1228, and (E) D1271.

Sample	D1153	D1228	D1271	D830 (RLD)
Polymer fraction (%)	34.4	53.8	46.4	51
API fraction (%)	36.9	31.8	40.1	33.2
Porosity fraction (%)	28.7	14.4	13.5	15.8

Table 1. Phase fractions of polymer, API and porosity determined from AI-segmented imaging data.

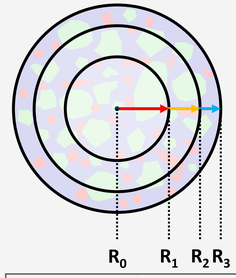

Figure 4a. Example cartoon of spatial/radial distribution of phases as the percentage of the phase within a given radius. For this cartoon, it is the percent of each phase within the given radius R_i.

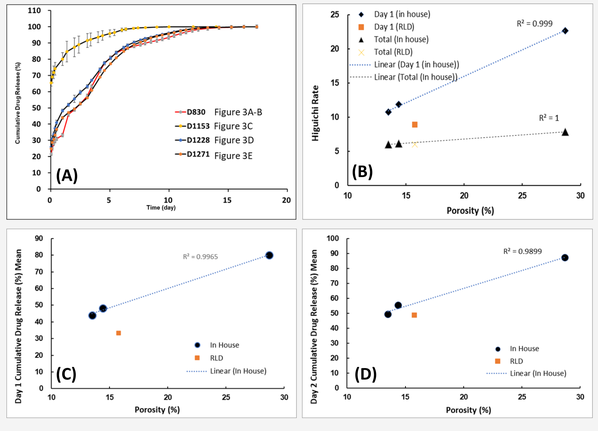

Figure 3. (A) *In vitro* release profiles for in-house and RLD samples. Reported data are mean ± std. dev. (N = 3) (B) Correlation of release rate with porosity for initial release (t = 1 day) and total release. Correlation of drug release with porosity after (C) 1 day and (D) after 2 days.

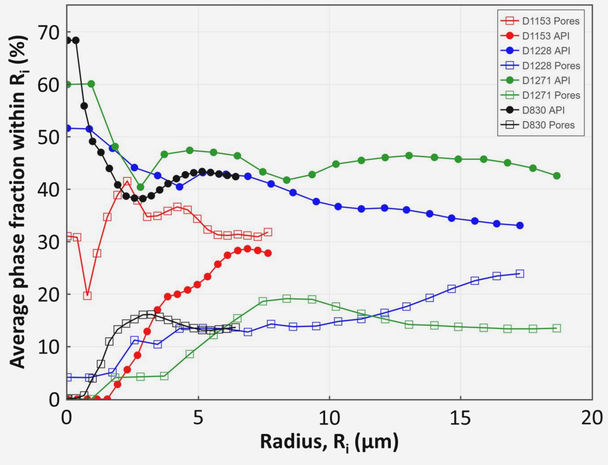

Figure 4b. Spatial distribution of API (circles) and pores (squares) of in-house samples (D1153 = red, D1228 = blue, and D1271 = green) and RLD (D830 = black).

CONCLUSIONS

- ❖High resolution FIB-SEM imaging combined with AI image analytics provides both visual and quantitative support to microstructure equivalence evaluations of PLGA microsphere formulations.
- ❖Microporosity was demonstrated to be a critical quality attribute that can significantly impact the drug release performance. It should be used to guide the formulation optimization process.
- ❖The quantified spatial distribution and its uniformity was demonstrated to have a correlation with release profile and may serve as a metric for assessing microstructure equivalence in PLGA microsphere formulations.
- ❖The demonstrated workflow has the potential to support generic drug development and regulatory assessment in microstructure equivalence.

ACKNOWLEDGMENTS

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