Assessing the Impact of Formulation on Microstructure Critical Quality Attributes in PLGA Microspheres

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Background and objective

The physical aging in polymer-based parenteral microspheres can significantly impact the therapeutic performance of the product. Aging produces changes in the microstructure of the microspheres. The specific microstructural changes induced by physical aging are therefore critical to identify as they may provide a mechanistic understanding of the product's life cycle. **In this study, both non-invasive X-ray imaging and high-resolution focused ion beam-scanning electron microscopy (FIB-SEM) are used to visualize the 3D microsphere microstructure. Artificial intelligence(AI)-based analysis is used to quantify the direct microstructural changes in the fresh and aged poly(lactic-co-glycolic acid) (PLGA) microspheres over multiple scales and provide a mechanistic understanding of changes in product performance.**

Materials and Methods

Risperdal Consta® (risperidone) microspheres were acquired from Janssen, where one population of microspheres were allowed to age at 4°C ambient conditions for 1 year prior to testing.

X-ray microscopy (XRM)

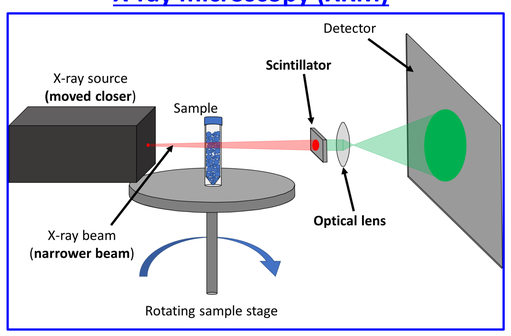


Figure 1. *Schematic diagram of XRM*

- XRM was used to non-invasively image the microspheres for characterization of the microsphere batch characteristics. A schematic is shown in **Figure 1** (left)
- 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 2** (right).

Focused ion beam SEM (FIB-SEM)

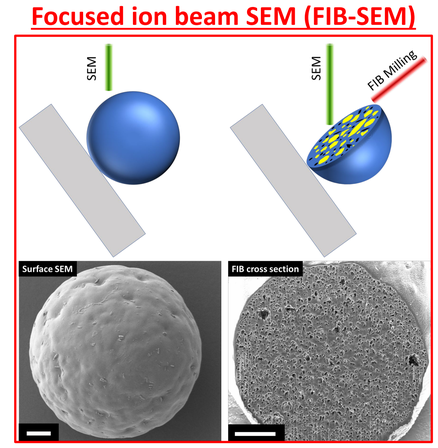


Figure 2. *FIB-SEM schematic and example images (scale bars = 10 µm)*

Images and Analysis

XRM density determination

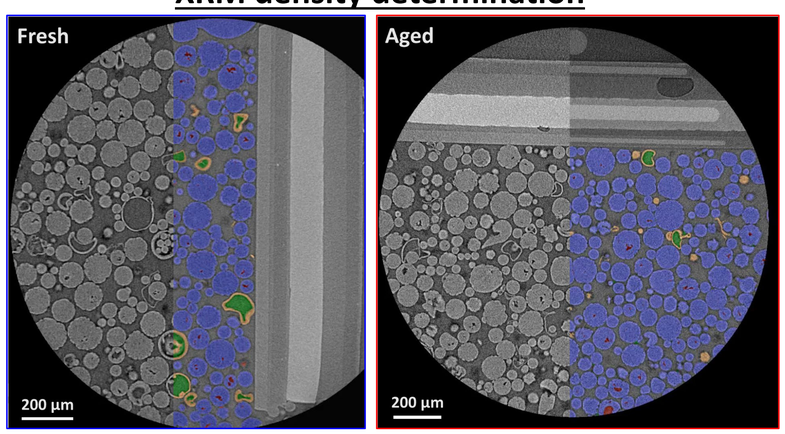


Figure 3. *Representative 2D slice from the XRM scans for the fresh and aged Risperdal Consta® microsphere samples, showing particles with different morphologies. The right side of the images shows the segmentation of the different particle types using machine learning and deep learning approaches.*

After identifying each microsphere, the density of every single sphere was determined using the polymeric calibrants seen in **Figure 3.**

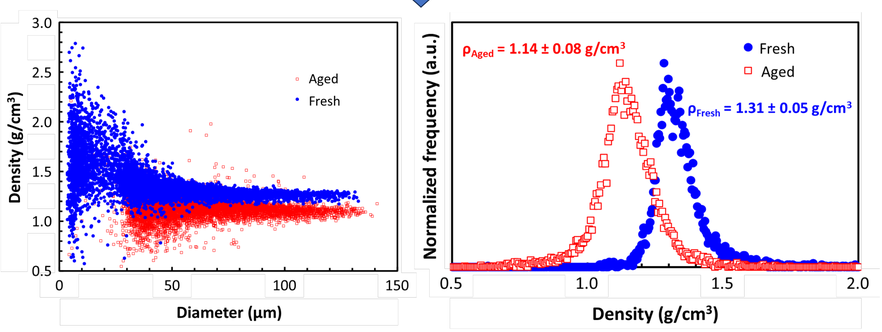


Figure 4. *The density as a function of microsphere diameter is plotted for both the fresh and aged sample (left plot), where the spheres above 25 µm in diameter show high uniform densities. The distribution of the microsphere densities is shown for the two samples (right plot) where a significant decrease in density is observed for the aged microsphere sample compared to the fresh sample.*

Hypothesis: Decrease in density → Increase in porosity
FIB-SEM can validate this hypothesis

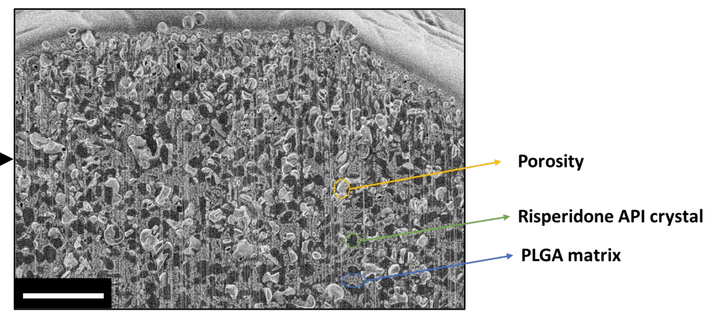
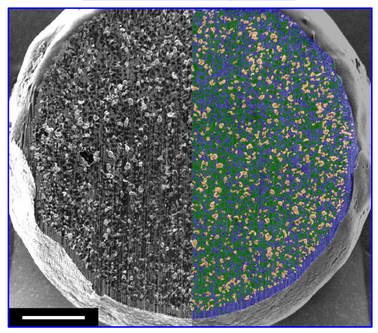


Figure 5. *High magnification FIB-SEM cross section used to identify the different material phases as indicated (scale bar = 5 µm)*

Fresh microsphere



Aged microsphere

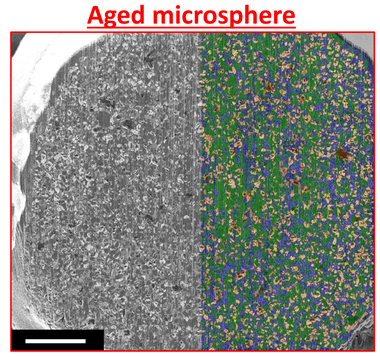


Figure 6. *Single slice from 3D FIB-SEM imaging for a fresh (left) and aged (right) microsphere, with the AI segmented phases shown on the right of the images, where blue = PLGA, green = risperidone crystals, and gold = porosity (scale bars = 10 µm)*

3D FIB-SEM determined porosity

Overview	Fresh	Aged
Resolution (µm)	0.03	0.03
Volume (µm³)	59 x 53 x 16	71 x 58 x 12
Porosity (%)	**18.9**	**29.7**
Risperidone (%)	**41.3**	**36.8**
PLGA (%)	**39.8**	**33.5**

Increase in porosity observed on 3D FIB-SEM consistent with XRM density determination.

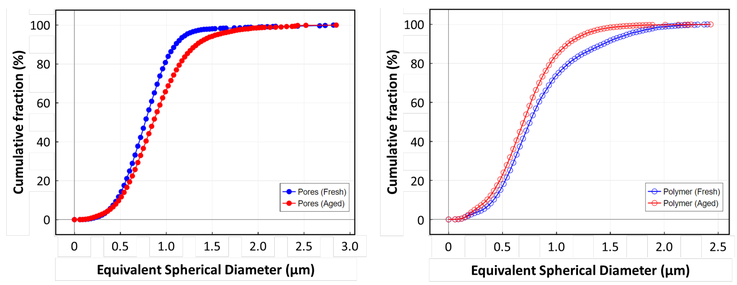


Figure 7. *Size distributions for the microsphere internal pores (left) and the polymer domains (right) for the fresh and aged microspheres, where the aged spheres show significantly larger pores and smaller polymer domains, consistent with XRM density determination.*

Conclusions

- Correlative XRM and FIB-SEM imaging were done to assess the impact of physical aging on the microstructure of Risperdal Consta® microspheres.
- XRM revealed that good density uniformity within each batch (intra-batch uniformity) while the aged spheres displayed a 14% decrease in density suggesting an increase in internal porosity as a function of aging.
- 3D FIB-SEM demonstrated an 11% increase in porosity with measurable changes in pore and polymer domain sizes consistent with an increase in porosity.
- XRM density analysis CAN be used to rapidly assess batch uniformity and internal microstructure.

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