Microstructural Characterization of Microsponge-Based Gel Products Using CryoSEM

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Abstract

Background and Purpose: Retin-A Micro® (tretinoin) topical gel, 0.1% (Retin-A Micro® gel) contains tretinoin loaded in porous microparticles referred to as "microsponges" that are dispersed in a gel vehicle. The microstructure of the products requires assessments at levels of both the drug loaded-microsponges and the gel matrices. Internal pore size of microsponges is an important microstructure characteristic that can be correlated to their drug loading capacity. This study aimed to develop sensitive imaging techniques to evaluate the microstructure of topical products and simultaneously, to assess the size and internal microstructure of microsponges in the gel matrices.

Methods: Retin-A Micro® gel products dispensed from both the tube and pump packaging configurations were first cryo-fixed in flush nitrogen. Then, morphology of the native and sublimed forms of the frozen, fractured samples was examined using a JOEL scanning electron microscope (SEM) (JSM7100F) equipped with a secondary electron detector. Microsponges pore size in Cryo-SEM images taken periodically during sample sublimation was calculated using the Image J software (NIH).

Results: The Cryo-SEM technique could successfully assess both the overall microstructure of the gel and the internal microstructure of the microsponges within the gel. From Cryo-SEM images of the native form of the gel, the size of microsponges and morphological information can be gained. Periodic imaging of gels that were undergoing sublimation showed morphological changes of the microsponges and the surrounding gel matrices. The sublimation process revealed the internal pores of the microsponges and left behind a unique pattern of integrated polymers in the gel system. The average internal pore sizes of the microsponges in gels dispensed from the tube and pump were calculated to be 18.57 nm^2 and 17.41 nm^2 at 90 minutes sublimation, respectively (n=3), which remained constant despite further sublimation till 210 minutes. After sublimation, the gel matrix dispensed from the tube exhibited visible hollow structures between polymeric filaments, while that was not observed in the formulation that was dispensed from the pump.

Conclusion: Here we introduce the use of Cryo-SEM techniques to evaluate the morphology and porosity of microsponges dispersed in a gel vehicle. A carefully designed protocol may be used to provide a means for comparing reference and generic products for their microstructural differences.

Introduction

Retin-A Micro® (tretinoin) topical gel, 0.1% contains tretinoin loaded in microsponges that are dispersed in a gel vehicle; it is marketed in two packaging configurations, a tube and a pump. Using microsponges in the formulation of tretinoin provides unique advantages such as an improved irritancy profile and enhanced photostability with increased drug loading. The microstructure of the microsponge gel products requires assessments at two levels: that of the drug loaded-microsponges and that of the gel vehicle. The purpose of this study was to develop sensitive imaging techniques that can reveal details of the inner microstructure of topical products and to also assess the size and internal microstructure of the microsponges simultaneously.

Materials and Methods

The Retin-A Micro® (tretinoin) topical gel, 0.1% product in a tube (Valeant Laboratories) was assessed along with the Retin-A Micro® (tretinoin) topical gel, 0.1% that is packaged in a pump (Oceanside Pharmaceuticals). Morphological examination of the internal microstructure of the gels in their native form was carried out using a JOEL scanning electron microscope (SEM) (JSM7100F) equipped with a secondary electron detector. For the cryo-scanning electron microscopy (cryo-SEM), the samples were loaded on the cryo-specimen holder and cryo-fixed in slush nitrogen (-210°C), then quickly transferred under vacuum to the cryo-preparation chamber in the frozen state. The frozen gel samples were fractured and then sputter-coated with platinum for 120 seconds at 10mA with Iridium. The coated samples were moved to the imaging chamber maintained at -145°C, equipped with an anti-contaminator which was maintained at -194°C. The imaging was undertaken at a voltage of 2 kV by collecting the secondary electron signal. To further understand the internal microstructure of the gels as well as the microsponges, the sample was sublimed in the imaging chamber at -80°C for a total of 3 hrs. The imaging was undertaken (uncoated) at various time points at a voltage of 2 kV by collecting the secondary electron signal. Image J software was used to calculate the internal pore size of the microsponges.

Results and Discussion

Cryo-SEM techniques developed and utilized to assess microsponge products demonstrated that both the overall microstructure and the internal microstructure of the Retin-A Micro® products from tube and pump configurations can be successfully assessed by Cryo-SEM. Figure 1A (Retin-A Micro® Tube) and 1D (Retin-A Micro® Pump) demonstrate the sublimed product (at a 5000X magnification) from which the microsponge size and spherical morphology information can be assessed.

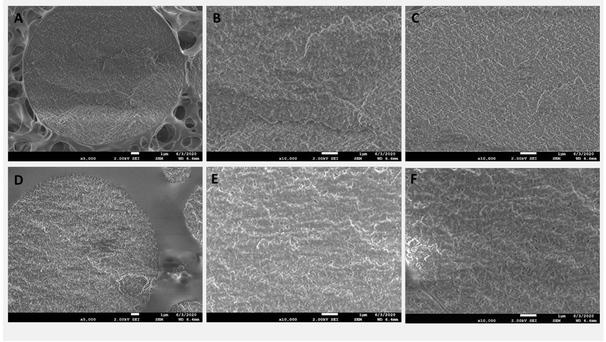

Figure 1. Cryo-SEM images of Retin-A Micro® Tube (A, B and C) and Retin-A Micro® Pump (D, E and F) microsponge products after sublimation illustrating the innate microstructure and internal microstructure post sublimation. **Images A and D were taken at 5000X magnification and images B,C,E and F were taken at 10,000X magnification. Scale bar 1 µm.**

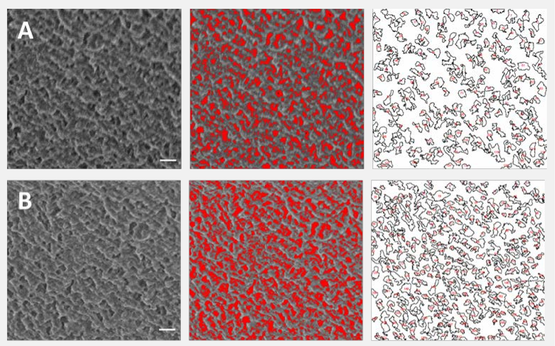

Figure 2. Representative cropped cryo-SEM images and Image J derived masks to calculate internal pore size of the microsponges from Retin-A Micro® Tube. **Scale bar 500 nm.**

Periodic imaging while the product is undergoing sublimation provides an opportunity to observe the water sublime from both, the gel and the microsponges, leaving behind a unique pattern of integrated polymers in the gel system. Figures 1B and C (Retin-A Micro® Tube) and Figure 1 E and F (Retin-A Micro® Pump) illustrate sublimation of the entire microstructure (including the microsponge) at 30 and 90 minutes, respectively. The sublimation procedure can reveal the internal pores of the microsponges. Assessment of the pore size of the microsponges is an important quality attribute that can be correlated to their drug loading capacity. Figures 2A and 2B demonstrate cropped images of a microsponge at 10,000 times magnification at 30 and 90 minutes respectively. The panels on the left are representative Cryo-SEM images, the panels in the center are image J-derived threshold maps, and the panels to the right are the area outlines of the pores. From these figures the internal pore size of the microsponges in the Retin-A Micro® Tube was observed to be 17.02±0.94 nm^2 and 18.57±1.2 nm^2 at 30 and 90 minutes sublimation, respectively (n=3) and the internal pore size of the microsponges in the Retin-A Micro® Pump was observed to be 16.15±1.2 nm^2 and 17.41±1.3 nm^2 at 30 and 90 minutes sublimation, respectively (n=3). Further sublimation at the same conditions up to 210 minutes did not result in any further change in pore size.

Figure 3 demonstrates the structure of the gel surrounding the microsponges in gel products dispensed from pump and tube. The tube product exhibited visible hollow structures between polymeric filaments following sublimation. By contrast, the pump product did not exhibit these hollow structures following sublimation.

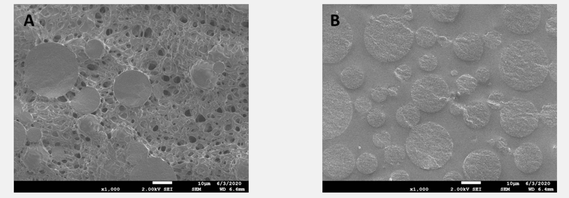

Figure 3. Cryo-SEM mages of Retin-A Micro® Tube (A) and Retin-A Micro® Pump (B) microsponge products illustrating the micro-structure of the bulk gel. The tube product exhibited intertwined polymeric filaments after it was sublimed, whereas sublimation of the pump product did not produce the hollow structures that enhanced the visualization of the polymeric filaments. **Scale bar 10 µm.**

Conclusion

This work has illustrated the application of cryo-SEM-based techniques to simultaneously characterize the size, morphology and internal microstructure (porosity) of microsponges - attributes that may be critical to the performance of a topical microsponge product, and which may be useful to compare in reference and prospective generic products.

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