Use of Optical-Laser-Scanning (OLS) Microscope to Assay Microparticle Morphological Changes

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Introduction

Poly(lactide-co-glycolide) (PLGA) has been used widely in the development of long-acting-injectable formulations. Our previous study has shown that PLGAs dissolve in different solvents depending on the lactide:glycolide (L:G) ratio [1]. The changes in morphological features of PLGA microparticles after exposure to different semi-solvents allows quantitative characterization of microstructural properties of the formulations. The goal of this study was to examine the microstructural changes after exposure to semi-solvents by laser-scanning microscopy.

Methods

PLGA microparticles, with and without naltrexone (NTX), were produced by an emulsion method using PLGAs with selected L:G ratios and their mixtures. PLGAs used were 50:50 (Evonik, RG504H), 75:25 (Evonik, 755S) and 100:0 (Akina, AP093). Samples of each particle type were chilled and exposed to toluene at 0 °C for 30 seconds. The particles were scanned using an Olympus OLS5000 confocal microscope before and after toluene exposure. The 3D scan images were assayed for width and roughness parameters using Olympus analytical software.

Results

The microparticles displayed a range of dissolution effects from the toluene. **Figures 1** shows 3D scan images of the particle types before and after toluene exposure. With the exception of PLGA 50:50, each microparticle type showed obvious morphological changes after toluene exposure, as observed in the 3D scans. The PLGA 100:0 particles “melted”, as did the PLGA 75:25 with and without NTX. The PLGA 50L +100L particles developed a “double-shell” structure after exposure to toluene. One of the most noticeable and measurable changes in the particles was the maximum width of the particles before and after toluene exposure, as shown in **Table 1**. The values shown are the average ± standard deviation of at least three particles prior to toluene exposure and at least 10 particles after toluene exposure. The PLGA 50:50 particles did not show a significant increase in width, further confirming the minimal impact of solvent seen in the 3D scans for those particles. All other particle types showed an increase in maximum width after solvent exposure. With the exception of PLGA 50:50, each microparticle type showed obvious morphological changes due to toluene exposure, as observed in the 3D scans in **Figure 1**.

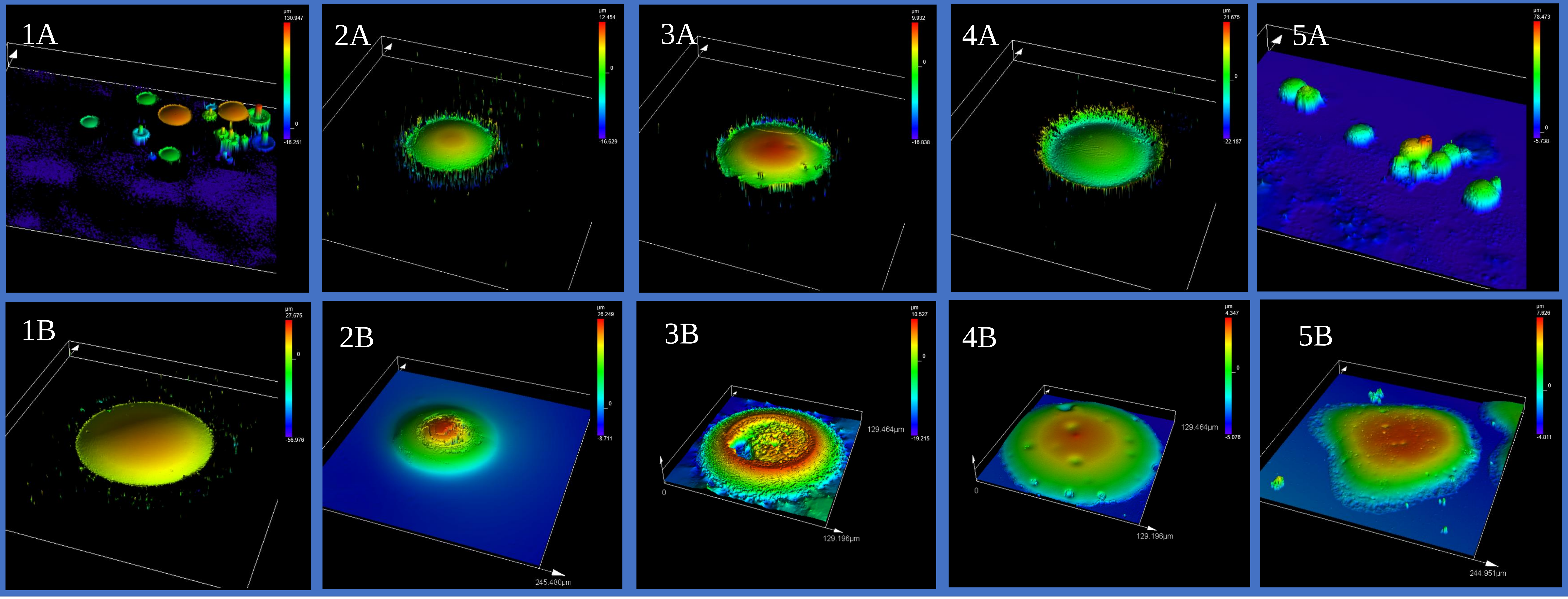

Figure 1. 3D scans of microparticles before (A) and after (B) exposure to toluene:
(1) PLGA 50:50,
(2) PLGA 100:0,
(3) PLGA 50L + 100L
(4) PLGA 75:25, and
(5) PLGA 75:25 + NTX.
Only the top portions of PLGA microparticles are shown.

The PLGA 50:50 particles did not show a significant increase in width, further confirming the minimal impact of solvent seen in the 3D scans for those particles. All other particle types showed an increase in maximum width after solvent exposure. Over 30 parameters are calculated with the LEXT software; however, due to space constraints, only three factors are shown here (**Table 2**): skewness (Ssk), core height (Sk) and Dale void volume (Vvv). The scans of each particle type were analyzed for all parameters before and after toluene exposure.

The roughness parameters were determined for each set before and after toluene exposure, for at least 3 and 10 particles, respectively. Skewness is a unitless parameter that evaluates deviations in the height distribution across the scan. The core height is the difference between the upper and lower levels of the core. The Dale void volume is an indicator related to portion of the material that is below the surface of the material. The roughness parameters require further evaluation and study to determine which parameters are key to defining solvent effect.

Table 1. Effect of toluene exposure on microparticle width.

Particle Type	**Maximum Width$_O$ (µm)**	**Number (N) of Measurements**	**Maximum Width$_f$ (µm)**	**Number (N) of Measurements**
PLGA 50:50	50.64 ± 13.65	7	67.26 ± 27.94	42
PLGA 75:25	45.00 ± 19.85	3	215.45 ± 102.61	10
PLGA 100:0	82.53 ± 6.63	3	212.69 ± 104.88	10
PLGA 50L+100L	82.06 ± 22.02	3	115.09 ± 43.96	35
PLGA 75L+NTX	26.63 ± 2.58	3	167.95 ± 52.02	10

Table 2. Effect of toluene exposure on selected roughness parameters.				
Particle Type	**Ssk**	**Sk (μm)**	**Vvv ($\mu m^3/\mu m^2$)**	**Number (N) of Measurements**
PLGA 50:50	1.08 ± 1.52	4.70 ± 2.24	0.19 ± 0.26	7
PLGA 50:50 after toluene	0.81 ± 0.54	16.83 ± 10.92	1.00 ± 0.68	42
PLGA 75:25	-0.39 ± 0.66	4.83 ± 2.68	0.09 ± 0.04	3
PLGA 75:25 after toluene	1.52 ± 0.42	21.54 ± 16.31	0.46 ± 0.34	10
PLGA 100:0	1.06 ± 0.02	7.68 ± 1.10	0.15 ± 0.03	3
PLGA 100:0 after toluene	1.15 ± 0.38	11.31 ± 7.2	0.27 ± 0.18	10
PLGA 50L+100L	0.92 ± 0.42	8.82 ± 2.58	0.65 ± 0.74	3
PLGA 50L+100L after toluene	-0.12 ± 1.29	27.31 ± 15.71	3.02 ± 2.64	35

Conclusion

An Olympus OLS5000 confocal microscope was used to examine 3D morphology of PLGA microparticles before and after exposure to toluene. The microparticles were exposed to toluene at 0 °C for 30 seconds. The parameters in Table 2 present a general overview of the changes of the outer surface of the microparticles.

Toluene was used as the first semi-solvent as it dissolves only the PLGAs with higher L:G ratios. A series of semi-solvents with different exposure times will be tested for in-depth characterization of particle microstructures.

Future studies will entail correlating these microstructural properties to product performance parameters, in particular drug release kinetics.

References

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