Supporting information

Direct Visualization of Drug Release in Injectable Implant by Laser Induced Breakdown Spectroscopy (LIBS)

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1. Materials

Unless stated otherwise, all chemicals were of reagent grade and purchased from Sigma-Aldrich (St. Louis, MO) or Thermo Fisher Scientific (Pittsburg, PA). Implants were obtained from Formulation at Merck & Co., Inc., Rahway, NJ, USA. Poly(ethylene vinyl acetate) (EVA: polymer name Ateva ® 1070, (9% vinyl acetate) was obtained from Celanese (Dallas, Texas).

2. *In Vitro* Accelerated Drug Release Experiments for Implants

***In vitro* drug release**: *In vitro* release was achieved by incubating implants (40% API content) in release media at elevated temperature with periodic media replacement to maintain sink conditions. The degree of drug release was monitored over time via sampling of the release media for assay by ultra-performance liquid chromatography (UPLC). At predetermined points (i.e., when approximately 25%, 50%, 75% and 100% release had been achieved) implants were removed from the media for analysis by LIBS & energy-dispersive x-ray spectroscopy (EDS). All implants removed from the release media were dried and stored with desiccant at 4°C prior to analysis.

Analysis of implants: After LIBS and EDS analysis, the remaining drug content of each individual implant was assayed to confirm the degree of drug release previously measured via media sampling. Implant sections were agitated in the extraction solvent at elevated temperature for 24 hours, ensuring complete release of any remaining drug. The drug content within the extraction solvent was assayed by UPLC.

Table S1. *In vitro* release profile and content uniformity data summary.

Label in the paper	25% Released	50% Released	75% Released	95% Released
Cumulative *in-vitro* Release in media	27.4 ± 3.8 % 16.5 ± 0.4 mg	54.1 ± 1.7 % 32.4 ± 0.8 mg	72.2 ± 2.6 % 43.3 ± 1.7 mg	94.2 ± 1.0 % 56.5 ± 0.6 mg
Post-Release Implant Assay	46.7 ± 1.9 mg	30.1 ± 0.6 mg	14.1 ± 0.4 mg	0.2 ± 0.0 mg
Total API Recovery	105.3 ± 3.8 % 63.2 ± 2.3 mg	104.2 ± 1.7 % 62.5 ± 1.0 mg	95.7 ± 2.6 % 57.4 ± 1.6 mg	94.5 ± 1.0 % 56.7 ± 0.6 mg

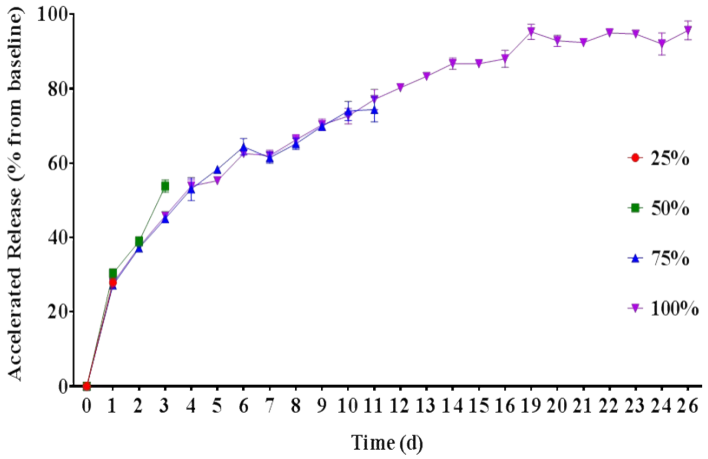


Figure S1. *In vitro* drug release profile of implants with 40 wt% API loading. This graph illustrates that all implants were releasing at the same rate even though, implants were stopped at different time points.

3. LIBS instrumentation

LIBS Instrumentation: Samples were analyzed using a J200 Tandem LA/LIBS system (Applied Spectra Inc., Fremont, CA, USA). The J200 consists of a 266 nm Nd:YAG laser (ns) operated with 10 Hz repetition rate, a Czerny–Turner spectrometer equipped with a CCD detector, and an x–y–z translational stage. Spot size, gate delay time, helium gas flow, and energy output were optimized for good signal/noise (S/N) ratio. For each parameter, a range of condition points were screened and the one which gave the highest signal was selected. All the related parameters, including the He flow rate, laser energy, repetition rate and spectrometer delay time, were optimized one by one. A 35 µm crater diameter (spot size) was used for all analyses (chosen for desired spatial resolution). The sample chamber was purged with 1.0 L min^{-1} helium gas. Gate delay = 0.01 µs; Energy output = 60 %; all parameters were controlled within Applied Spectra's Axiom Software.

Sample Preparation and Analysis: Implants were cut into sub-millimetre sections, and placed into the laser chamber. The implant samples were analyzed using 60 parallel lines, where a spatial resolution of 440 × 60 was achieved over a 2.2 mm × 2.2 mm area with implant sample in the middle. The intensity maps of F 685.6 nm in different implants were successfully generated. Calibration was built on a control implant with 35 wt% API loading. Data analysis was performed using Applied Spectra's Aurora Data Analysis Software package.

Calibration method: Due to the limited variety of samples, an implant with 35 wt% API loading was utilized as one-point calibration standard. One line pattern was designed on the standard sample with a length of 1.8 mm. All the conditions are the same as used for real measurements. 360 data points were collected for the standard. The results were background corrected, averaged and then utilized to quantify the concentration of API in real samples. To verify this method, the bulk analysis result (the average of all the data points after quantification) was correlated with the traditional HPLC results from in vitro drug release experiment. The correlation factor was found to be 0.9866, suggesting the high accuracy this calibration method. LOD (3 * standard deviation of the blank) can also only be estimated using a single-point curve, which was 0.08 wt.%.

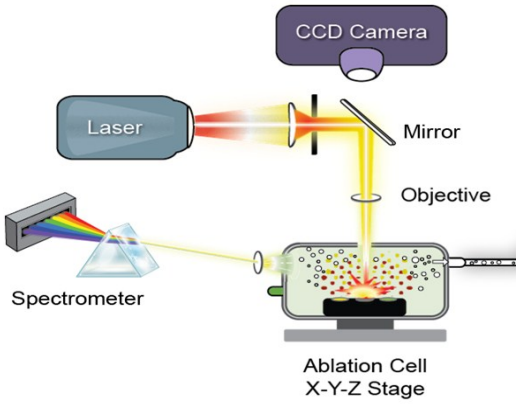


Figure S2. The scheme of LIBS instrumentation.

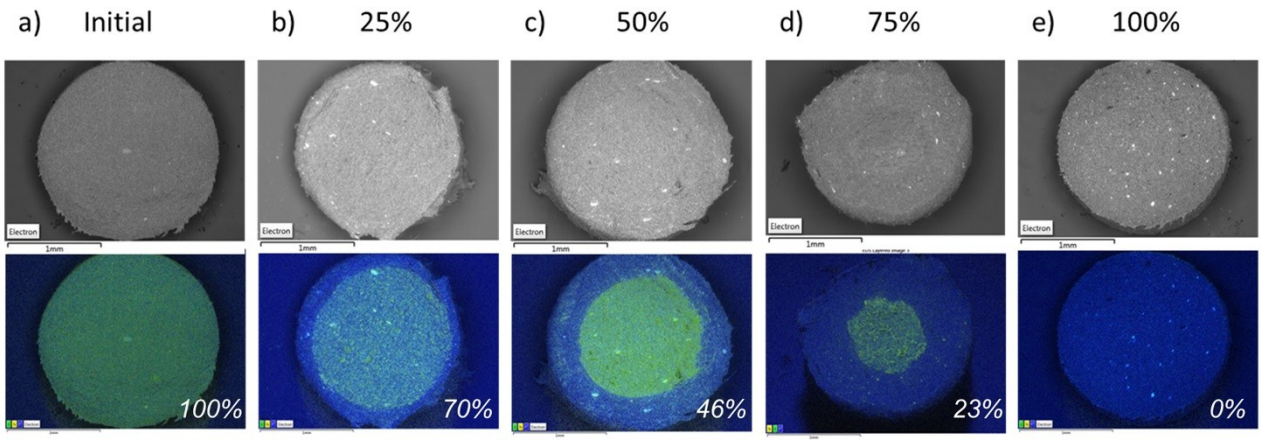
Table S2. Experimental conditions of LIBS.

Instrumental Variable	Optimal Parameter
Laser wavelength	266 nm
Repetition rate	10 Hz
Energy per pulse	5 mJ
Spot size	35 µm
Energy output	60%
Gate delay	0.01 µs

He flow	1 L/min
F wavelength	685.6 nm

4. SEM/EDS Instrumentation

Scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) imaging: To investigate drug distribution, implants were imaged using scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) at predetermined time points (initial, 25%, 50%, 75% and 95%). Image analysis and capture was performed using a Hitachi SU5000, equipped with a backscattered electron detector, and an Oxford Instruments Xmax 80 silicon-drift detector for EDS analysis. Implants were sectioned with a razor blade, mounted on carbon conductive tape, and then loaded into the SEM chamber. To prevent charging of non-conductive materials, implant sections were imaged in a reduced vacuum pressure (50 Pa), with a working distance of 15 mm. Electron images were acquired using the back-scattered electron (BSE) detector, with the accelerating voltage and spot intensity set to 12



kV and 70 respectively. Electron images were scanned at 4096 resolution, pixel dwell time 10 µs, 1 frame. For EDS mapping: 4096 resolution, 16 frames, 10 keV cut-off, 2048 channels, process time 5 min, pixel dwell time 10 µs. The following elemental analysis was used for chemical mapping: EVA (C), and API (N, F, C). The quantitation of the *Unreleased*% was also performed for each EDS image.

Figure S3. Electron images and EDS images of implants at different stages of API release. Top row represents electron images and bottom row EDS images. a) Initial. b) 25 % API release. c) 50 % API release, d) 75% API release and e) 95% API release. The area percentage of unreleased API, termed *Unreleased*%, is shown in the bottom right of each map. Notes: F in green, N in yellow, and C in blue.

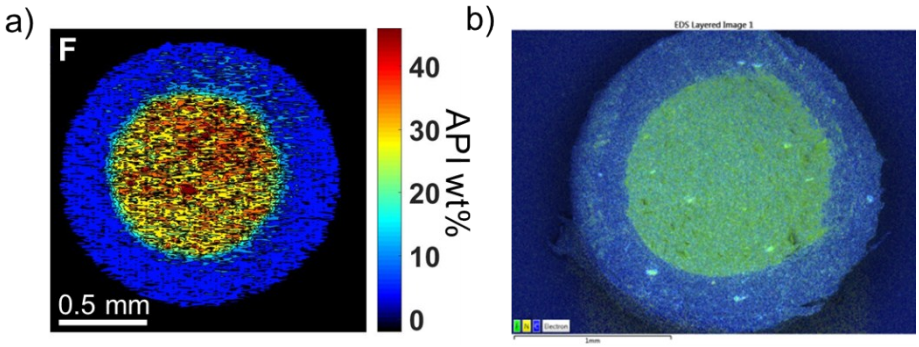


Figure S4 Comparison of a) LIBS imaging and b) EDS imaging for implant with 50 % drug released. Notes: F in green, N in yellow, and C in blue.

5. Drug Release Uniformity in the Whole Implant

Since the implant has a large length (4.0 cm), it is important to investigate the uniformity throughout the entire implant. The implant with 50% API release was taken as an example. Five sections at different position of the rod (the distance to the end is 0, 0.5, 1.0, 1.5, and 2.0 cm, respectively) were measured (Figure S5). As shown in Figure S6, the first section demonstrated lower concentration of API, which can be ascribed to the drug release in both directions of along the length and perpendicular to the length. From sections at a distance of 0.5 cm to the very middle section (2.0 cm), both the concentration and the distribution of API are very similar to each other, suggesting the high uniformity of the drug release process.

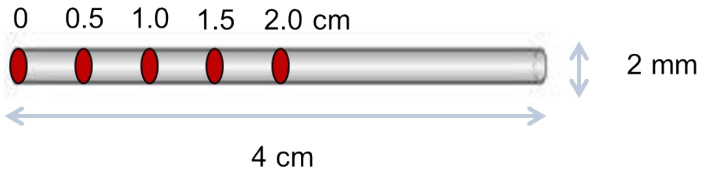


Figure S5. Thin sections (100 µm) were taken from the implant at different positions. The red oval indicates the positions (the number shown indicates the distance between the sample positions to the left end of the implant).

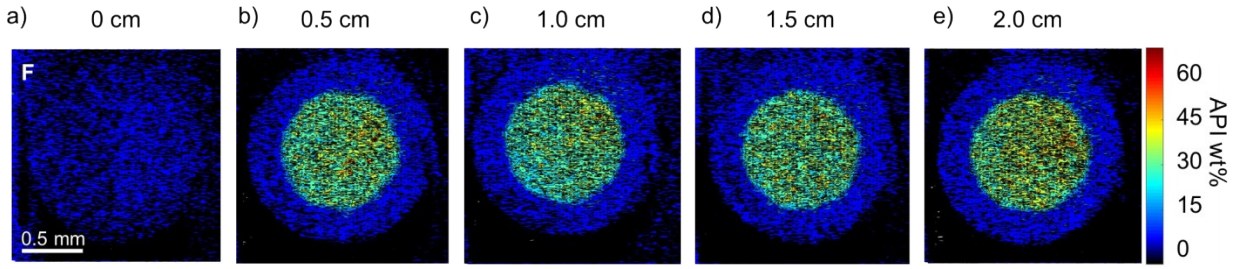


Figure S6. Concentration images of implant at different positions of API release by LIBS. From left to right shows imaging at different depth: a) 0 cm. b) 0.5 cm. c) 1.0 cm. d) 1.5 cm and e) 2.0 cm.

$$Unreleased\% = \frac{Spatial\ Area\ Containing\ Center}{Total\ Area\ of\ Implant} =$$

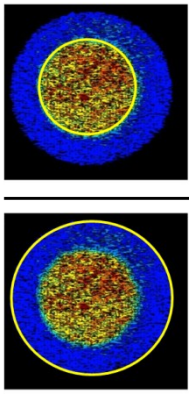


Figure S7. The definition of *Unreleased* %, which is the area percentage of the center area in the whole implant area.

Table S3. The comparison of *Unreleased*% between LIBS and EDS .

Implant API Release Stage	***Unreleased% by LIBS***	***Unreleased% by EDS***
Initial (0%)	100	100
25%	70	70
50%	39	46
75%	13	23
100%	0	0