Experimental and computational understanding of pulsatile release mechanism from biodegradable core-shell microparticles

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Next-generation therapeutics require advanced drug delivery platforms with precise control over morphology and release kinetics. A recently developed microfabrication technique enables fabrication of a new class of injectable microparticles with a hollow core-shell structure that displays pulsatile release kinetics, providing such capabilities. Here, we study this technology and the resulting core-shell microstructures. We demonstrated that pulsatile release is governed by a sudden increase in porosity of the polymeric matrix, leading to the formation of a porous path connecting the core to the environment. Moreover, the release kinetics within the range studied remained primarily independent of the particle geometry but highly dependent on its composition. A qualitative technique was developed to study the pattern of pH evolution in the particles. A computational model successfully modeled deformations, indicating sudden expansion of the particle before onset of release. Results of this study contribute to the understanding and design of advanced drug delivery systems.

INTRODUCTION

Biodegradable core-shell microparticles represent a promising class of biomaterials with applications that can improve efficacy and adherence, which is particularly important for patients in a developing world. These applications include single-injection vaccine delivery, transdermal vaccine delivery, cancer immunotherapy, and pH-triggerable oral drug delivery, all of which are otherwise infeasible with traditional drug delivery systems (1–3). Controlled drug delivery technologies can particularly aid in the treatment of diseases that require multiple shots by improving patient adherence (4–6). Patient adherence is reported to be as low as 50% globally, costing the U.S. health care system alone more than $100 billion annually (7–9). They can also potentially unlock new therapies by removing the need for multiple surgeries or difficult injections in the treatment of solid tumors (10–15). Furthermore, by simplifying the dosing regimen and eliminating the need for multiple visits to health care providers, a key global challenge especially in a developing world with poor health care infrastructure, single-administration systems can also be an effective tool in the eradication of infectious disease outbreaks (16).

Sustained drug delivery microparticles have been widely studied in the literature (17–19). However, the mechanism of pulsatile release from microparticles upon degradation is poorly understood. The mechanism of degradation in biodegradable drug delivery systems is perceived to be a complex phenomenon, caused by an interplay between multiple factors, such as pore formation, swelling, pH change, change in the glass transition temperature, and buffer microenvironment (17–19). Understanding the mechanism of release from biodegradable microparticles with complex geometries is crucial for the design of next-generation biomaterials (20, 21). It can also enhance our understanding of conventional drug delivery systems such as microspheres made from poly(lactic-co-glycolic acid), PLGA (17–21). Comprehensive studies have been conducted on biodegradable microspheres (17–21). However, the mechanism of degradation in microstructures fabricated by emerging microfabrication techniques such as high-precision three-dimensional (3D) printing or advanced soft lithography has been rarely investigated. As the complexity of biopharmaceutical products increases, the need for such sophisticated drug delivery systems becomes increasingly evident.

To provide insights into the pulsatile release mechanism from biodegradable core-shell microparticles, we performed a comprehensive study using various experimental and computational tools. Specifically, we focused on particles with a 3D microstructure that can be microfabricated or 3D printed thanks to a multilayer microfabrication technique termed StampEd Assembly of polymer Layers (SEAL) (1). This technique is a high-resolution, bottom-up approach compatible with medical-grade materials such as PLGA (1). In brief, core-shell microparticles are fabricated first by filling the aqueous solution of therapeutic cargo into the core inside the base layer. Upon dispensing, the aqueous phase in the drug solution instantaneously evaporates, leaving a completely dry core filled with the therapeutic cargo precipitated in solid form. Subsequently, an additional layer of particle cap is aligned and sintered with the filled base, fully sealing the core and forming a 3D core-shell microstructure (Fig. 1A) (1, 2). These 3D core-shell microparticles demonstrate a delayed pulsatile profile, which can be programmed from a few days up to several months (1, 2). These microparticles’ unique tunability, in terms of release time point, morphology, and compatibility with biologics and hydrophilic small molecules, provides a wide range of opportunities for pulsatile drug delivery (1, 2).

Note that each individual microparticle provides one pulse of the released cargo. A population of particles therefore can provide either the same pulsatile release kinetics or multiple pulses. Particles...
with the same design are expected to release the same day, providing one pulse, while multiple particle types lead to multiple pulses, depending on their design. Because of the hollow structure of the particles, they are called core-shell microparticles (not to be mistaken with core-shell microcapsules). The pulsatile kinetics is defined as a release profile with an obvious delayed onset and a tight release window with respect to the onset timeframe.

In this study, we first investigate the microstructural evolution occurring on the surface and within the internal structure at a single-microparticle level. We use multiple tools including optical microscopy (OM), surface and cross-sectional scanning electron microscopy (SEM), energy-dispersive x-ray spectroscopy (EDS) in conjunction with SEM, and state-of-the-art nanoresolution computed tomography (nanoCT). We also use differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and compression tests to study the change in material properties of particles upon degradation. Next, using fluorescent-based labeling and nanoresolution 3D printing (NanoScribe), complementing the SEAL method, the effect of a wide range of size- and material-related parameters on release profile is directly studied. A qualitative method is developed to study pH change in various particle groups and examine parameters affecting the deformation of particles. We also study how addition of polyethylene glycol (PEG) to PLGA modulates the release time point and affects pH. To reinforce experimental understanding, a Multiphysics finite element model is further constructed on the basis of the theory of poroelasticity. Studying the effect and significance of four dimensionless parameters on particle deformation is not feasible with a purely experimental approach. To overcome this limitation, we coupled the numerical model with a design-of-experiment (DOE) study. Overall, these tools allowed us to form a mechanistic understanding of pulsatile release from 3D-printed or SEAL-fabricated biodegradable core-shell microparticles having a hollow microstructure.

Fig. 1. Visualization of deformation and pore formation in biodegradable core-shell microparticles. (A) Schematic of the key components of a core-shell microparticle system and the fabrication process for making these particles. (B) Visualization of microstructural evolution in an empty core-shell microparticle with fast-degrading PLGA in the cap (RG 502H) and slow-degrading PLGA in the base (RG 756S) with an empty 4X core at different time points obtained by (B) OM, (C) surface SEM, (D) cross-sectional SEM, and (E) nanoCT visualization between comparable cross sections of the particles. (F) Change in porosity of microparticle over time obtained from nanoCT analysis. ns, not significant. (G) Change in glass transition temperature (normalized by day 0) of polymer films as a model for fast-degrading cap (RG 502H). The actual average values of glass transition temperatures in degrees Celsius are shown at the top/bottom of each data point in the graphs, with buffer temperature of 37°C. Scale bars, (A to D) 50 µm and (E) 100 µm. Error bars show the standard error of the mean. Please refer to movies S1 to S4 for 3D scanning of nanoCT imaging for the particles shown in (E). In this figure, (*) and (**) correspond to a P value of less than 0.05 and 0.001, respectively. Photo credit: Morteza Sarmadi, Massachusetts Institute of Technology.
**RESULTS**

**Investigating microstructural evolution upon degradation**

To investigate the evolution of the internal structure upon hydrolysis-based degradation, various assays were performed on a model cubic core-shell microparticle with cap and base made from PLGAs of different molecular weights (MWs). The cap and base were fabricated from a fast-degrading PLGA (RG 502H) and a slow-degrading one (RG 756S), respectively. We used various tools, namely, OM, cross-sectional and surface SEM, EDS-SEM, and nanoCT, to study the mechanism of degradation (Figs. 1 and 2 and movies S1 to S4). OM revealed that, unlike the slow-degrading base, which remains mostly transparent up to day 10, upon hydrolysis, the fast-degrading cap starts to turn opaque 1 day after incubation. This change in the transparency of the cap was accompanied by deformation and initial expansion, while the base maintained its transparency and cubic microstructure (Fig. 1B). Surface SEM indicated deformation patterns on the cap, most notably 2 days after incubation, which were more pronounced on the edges (Fig. 1C). Results of cross-sectional SEM and nanoCT imaging demonstrated the creation of a pore-forming zone on the edge of the cap, while the base remained fully nonporous (Fig. 1, D and E). Networks of interconnected pores were observed at day 10, connecting the core to exterior buffer. Quantification of porosity (void in cap and core combined) based on nanoCT imaging for individual particles revealed that the cap became significantly more porous at day 10 (~3 times more than at day 7) while having similar porosity at days 3 and 7 (Fig. 1D). Observations made by OM and SEM were confirmed across particles tested (figs. S1 to S3). The same observation regarding degradation of the cap and pore progression was confirmed in other cubic core-shell microparticles with a similar polymer composition but a larger core (18 nl versus 4 nl) and a thinner cap (50 μm versus 100 μm) (fig. S3).

To investigate the effect of degradation on material properties of SEAL-fabricated microparticles, we further performed DSC, TGA, and mechanical compressive tests. Tests involved studying the change in glass transition temperature ($T_g$), degradation temperature ($T_d$), and elastic modules ($E$) of the samples made from RG 502H over time ($n = 3$ to 5). Results suggested a decrease in all three properties upon degradation by hydrolysis, more significantly after 9 to 10 days (Fig. 1G and figs. S4 and S5). Notably, it was previously shown that day 10 was also associated with significantly higher porosity (Fig. 1, D to F, and fig. S2A). The values of $T_g$, $T_d$, and $E$ dropped by more than 30%, 10%, and two orders of magnitude from days 0 to 10, respectively.

To visualize release, Alexa Fluor (AF)–labeled 10-kDa dextran (10kD-dext), a model biomacromolecule, was encapsulated in the core, and OM was used to visualize its release from the core-shell microparticles at different time points (Fig. 2A). OM results revealed that cargo remained fully entrapped in the core up to 5 days, but it started to leave the particle through the cap on day 6 (Fig. 2A). Notably, the cargo traveled through the pore networks previously identified (Fig. 1, D and E) within the cap (Fig. 2B). We then encapsulated iron(II) sulfate heptahydrate (FeSO$_4$·7H$_2$O) in the core to further study the mechanism of release with EDS-SEM using elemental analysis (Fig. 2C). In agreement with previous observations, EDS-SEM also confirmed the entrapment of cargo at day 3 and the formation of a network of Fe (red contour) in the porous cap, connecting the core to exterior buffer at day 10 ($n = 3$) (Fig. 2C and fig. S6).

**Effect of design parameters on release kinetics**

The effect of size-related and material-related design parameters on the release kinetics was studied (Fig. 3). Size-related parameters included cap thickness, core size (volume), particle geometry, wall thickness, and molecular size of the cargo. The range of cargo MWs included free AF647 and AF647 conjugated to dextran of various MWs: 10, 70, and 2000 kDa. Material-related parameters included PLGA MW and end group (i.e., PLGA terminated with ester or acid end groups). We also studied release kinetics of hybrid caps, composed of a polymer blend of two PLGAs with varied MWs. The release time point was defined as the earliest day at which at least 50% of the cargo is released.

As seen in Fig. 3, the overall delayed pulsatile release profile was maintained across all 25 cases of particle designs studied. Increasing

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the cap thickness twofold, from 50 to 100 μm, while maintaining the particle composition resulted in a maximum difference of only 2 days in release time point across the entire range of cargo molecular sizes studied (Fig. 3, A, B, and G). The same difference in release time point was observed when increasing the core size 4.5 times, from 4 nl (200 μm by 200 μm by 100 μm; 4X) to 18 nl (300 μm by 300 μm by 200 μm; 18X), with identical particle composition (Fig. 3, B, C, and H). Similarly, among a given group of particle dimensions, varying MWs of the cargo led to a maximum difference of 2 days across the range studied (Fig. 3, A to C, G, and H). Statistical analysis of release time point comparing the effect of (G) cap wall thickness, (H) core size, (I) shape, (J) cap MW and end group, and (K) different blends of PLGAs in the cap. (L) SEM images of the base in a group of cubic and cylindrical microparticles studied in (D) and (I) fabricated by NanoScribe 3D printing. All release profiles correspond to particles incubated in vitro (n = 6 to 10). Release time point corresponds to the earliest day for average cumulative release of at least 50% of the cargo. Scale bars, 100 μm. In this figure, (*), (***), and (****) correspond to a P value of less than 0.05, 0.001, and 0.0001, respectively. Error bars show the standard error of the mean. Photo credit: Morteza Sarmadi, Massachusetts Institute of Technology.

Fig. 3. Investigating the effect of design parameters on release kinetics in biodegradable core-shell microparticles. (A) Cumulative release profile of dextran-free AF647 and AF647-labeled 10-kDa, 70-kDa, and 2000-kDa dextran from core-shell microparticles with different geometries including (A) 4X-core, 100-μm-thick cap, (B) 4X-core, 50-μm-thick cap, and (C) 18X-core, 50-μm-thick cap. (D) Release profile of AF647-labeled 10-kDa dextran from core-shell microparticles with different shapes and wall thicknesses with a 1X core. Particles in (A) to (D) are made from the same fast-degrading cap and slow-degrading base as in Figs. 1 and 2. Cumulative release profile of AF647-labeled 10-kDa dextran from core-shell microparticles with caps made from (E) pure PLGAs or (F) a blend of two different PLGAs. Particle base in (E) and (F) is made from the same slow-degrading PLGA for the rest of this study (RG 756S). Statistical analysis of release time point comparing the effect of (G) cap wall thickness, (H) core size, (I) shape, (J) cap MW and end group, and (K) different blends of PLGAs in the cap. (L) SEM images of the base in a group of cubic and cylindrical microparticles studied in (D) and (I) fabricated by NanoScribe 3D printing. All release profiles correspond to particles incubated in vitro (n = 6 to 10). Release time point corresponds to the earliest day for average cumulative release of at least 50% of the cargo. Scale bars, 100 μm. In this figure, (*), (***), and (****) correspond to a P value of less than 0.05, 0.001, and 0.0001, respectively. Error bars show the standard error of the mean. Photo credit: Morteza Sarmadi, Massachusetts Institute of Technology.

AF647-labeled 10-kDa dextran in a cubic particle. The particle was made from a 100-μm-thick cap, 4-nl core, and RG 756S base. It was revealed that the difference between the average release time points for microparticles capped with PLGA of 19 kDa, either ester-terminated (RG 502) or with acidic end group (RG 502H), was as large as 16 days (Fig. 3, E and J). The difference between the average release time points of two ester-terminated PLGAs with varied MWs (502 and 505 at approximately 19 and 53 kDa, respectively) was found to be 7 days. We also studied the release kinetics in hybrid caps composed of different PLGA blends with varied MWs and/or end groups. The average release time point in hybrid caps composed of 502 and 505 at approximately 19 and 53 kDa, respectively) was found to be 7 days. We also studied the release kinetics in hybrid caps composed of different PLGA blends with varied MWs and/or end groups. The average release time point in hybrid caps composed of 502H and either 502 or 505 (X) was statistically insignificant for 502H:502 75:25 and 502H:505 50:50. The difference was found to be significant when the ratio of 502H:X changed to 25:75. Collectively, these results highlighted that PLGA MW and end group had a more substantial impact, compared to particle size and shape, in controlling the release time point.

We also compared the release kinetics in heterogeneous and homogeneous core-shell particles. In the homogeneous particles, both
cap and base were fabricated from the fast-degrading PLGA (RG 502H). In the heterogeneous group, only the cap was made from 502H, and the base was fabricated from the slow-degrading PLGA (RG 756S). Aside from material composition, other design parameters were identical among the two groups. The release time point was similar among both groups, showing that degradation was dominated by the fastest degrading polymer (fig. S7). Moreover, the release time point could be controlled by only changing the cap composition (instead of changing the entire particle composition).

Cross-sectional SEM in the homogeneous group revealed that pores started to progress from the edges of the particle toward the core, eventually making a patch of interconnected pores at day 10 (fig. S7). The pore progression starting from the edge of the particle was found to be similar to the degradation pattern in heterogeneous particles with only the cap made from the fast-degrading PLGA (Fig. 1D).

pH evolution upon degradation
To study dynamic pH changes in particles, we used a qualitative colorimetric technique with the same particle design studied in Figs. 1 and 2 (Fig. 4A and fig. S8). Initially, a universal pH indicator (pH 4 to 10) was dispensed into the core, and color change was visualized over incubation time in phosphate-buffered saline (PBS) at 37°C (Fig. 4A and fig. S8). We observed that upon water permeation into the core, starting at day 1 (fig. S8), the indicator changed color from a spectrum of yellow to red, demonstrating a minimum pH of 4 or less (Fig. 4A). The pH change in the cap was studied by OM in two groups loaded with another low-pH indicator, thymol blue (TB): cap only before sealing onto the base (Fig. 4B) and a sealed cap imaged after attaching it to the base (Fig. 4C). The control group was a sealed cap and base structure and no indicator loaded (Fig. 4F). OM results demonstrated the formation of a less acidic zone on the edge of the cap (yellow; 2.8 ≤ pH ≤ 8.0), progressing toward the center of the particle, while the center of the cap remained more acidic (pink; pH ≤ 1.2). Comparison between the cap-only and full cap-base particles indicated that acidic products, formed upon degradation, were present in the core (pink center in Fig. 4, B and C), while the cap-only group turned entirely yellow (less acidic) at day 5. Next, the effect of PEG (2 kDa; PEG/PLGA, 50:50) on pH when doped in the cap was studied, making a blend of PEG/PLGA, with a full sealed particle (Fig. 4D) and a cap-only structure before sealing (Fig. 4E). We hypothesized that addition of PEG could accelerate pore formation in the cap, leading to higher pH in the cap caused by improved clearance of acidic products. Both PEG-loaded PLGA caps remained pale yellow and without any highly acidic pink region, for up to day 6, at which point the pH indicator diffused out from the cap. Observations regarding pH evolution were further confirmed in an additional three particles per group, per time point (fig. S8).

We analyzed the normalized projected area (from the top view) of the cap in each group in the pH study (n = 3 to 5) (fig. S10). The projected area (normalized by day 0) followed different trends, with PLGA-only groups showing expansion, while caps containing PEG decreased in size over time. The extent of expansion or shrinkage was projected area (normalized by day 0) followed different trends, with PLGA-only groups showing expansion, while caps containing PEG decreased in size over time. The extent of expansion or shrinkage was

To better understand the effect of PEG loading, surface SEM was used to visualize the microstructural evolution of sealed cap with pure PLGA or PEG/PLGA caps (Fig. 4, G and H) in multiple particles (additional particles in fig. S11). As shown, the PEG-loaded group represented more pronounced pore formation on the surface combined with faster breakdown of the cap, being considerably peeled off the base at day 5. Conversely, the PEG-free group represented expansion with a less pronounced pore formation in comparison. We further realized that the enhanced pore formation in particles with a PEG-loaded cap led to earlier release of the cargo compared with particles with a PEG-free cap (day 3 for 2-kDa and day 4 for 20-kDa PEG in PEG/502H) (Fig. 4I). However, this observation held only when PEG was physically doped into the cap, not when a block copolymer PEG-b-PLGA with a PEG segment of similar MW (2 kDa) to free PEG was used. Change in release time point in the latter was found to be statistically insignificant from the control group (PEG-free 502H) (Fig. 4I).

Computational modeling of particle deformation
To reinforce our experimental understanding, a numerical multiphysics model based on the theory of poroelasticity was constructed using COMSOL Multiphysics. The model coupled solid mechanics formulations to Darcy’s law (flow in porous media) (Fig. 5A). Subsequently, we modeled 2D geometry of a cubic core-shell microparticle at the central cross section, with the same dimensions as in the experiments (Fig. 5B). During the simulations, the average vertical displacement in the cap (Vmean), as an indicator of cap deformation, was computed (see the Supplementary Materials). Boundary conditions were specified as both cap and base made from porous linear elastic materials, with specified material properties such as elastic module (E), porosity (ε), and permeability (κ) (Fig. 5C). Two types of pressure were considered: external pressure applied on the cap (Pout) and an internal pressure in the core (Pin). It was also postulated that water enters from the edge of the cap (at a velocity of vout) and enters into the core (at a velocity of vin) as qualitatively observed in the experiments (fig. S8). Structure of the meshed model was chosen as physically optimized (Fig. 5D). Additional assumptions (described in the Supplementary Materials) were made to further enable the feasibility of the simulations.

To study the effect of key parameters in poroelasticity on the particle deformation, an L18 orthogonal DOE array was constructed on the basis of four dimensionless parameters (table S1). These parameters include (i) the ratio of Young’s modulus of base/cap (Ebase/Ec), (ii) the ratio of permeability of cap/base (κc/κbase), (iii) the ratio of permeability of cap/base (λ = κc/κbase), and (iv) the ratio of external/internal pressure (Pout/Pin). Simulations were performed for four different ratios of cap/base porosity (Rc = εc/εbase), resulting in a total of 52 simulations. We investigated the deformed shape and vertical displacement of the cap (Vmean) as two main outputs in this study. A purely solid mechanics (nonporous) model and a full multiphysics model (porous) were analyzed. Comparing these models helped us understand how the effect of these parameters would change in the absence and presence of pores in the system.

The deformation of the cap started from the center and progressed toward the edge and exterior parts of the cap (Fig. 5E). The nonporous model yielded multiple different mode shapes depending on simulation conditions (Fig. 5F). However, all mode shapes in the full poroelastic model followed two classes of deformation
Fig. 4. pH evolution in biodegradable core-shell microparticles. (A) Water permeation into the core over time leading to change in the color of the pH indicator loaded in the core (4X). Color change over time in various groups, with TB loaded in the cap (yellow; 2.8 ≤ pH ≤ 8.0; and pink, pH ≤ 1.2), in different groups, namely, (B) cap-only (pure PLGA), (C) sealed-cap (with pure PLGA cap), (D) full cap-base (PLGA/PEG cap), (E) cap-only (PLGA/PEG), and (F) full cap-base particles (no TB in cap), as the control group. Surface SEM comparison of change in the microstructure of core-shell microparticles with (G) PEG-free and (H) PEG-mixed [2-kDa PEG, 5% (w/v)] caps upon incubation in buffer. Effect of addition of PEG to the PLGA in the cap on release time point in (I) PEG (2 kDa) physically doped into the cap PLGA or (J) PEG added through PEG-b-PLGA block to the cap PLGA (PEG, 2 kDa; PLGA, 11 kDa). Scale bars, 50 μm. Bases are made from slow-degrading PLGA (RG 756S), and, except the group in (A), the core is initially empty. PLGA used in the cap (not conjugated with PEG) is fast-degrading (RG 502H). Error bars show the standard error of the mean. In this figure, (****) corresponds to a P value of less than 0.0001. Photo credit: Morteza Sarmadi, Massachusetts Institute of Technology.
Fig. 5. Computational modeling of deformation in biodegradable core-shell microparticles. (A) Conceptual overview of the multiphysics simulation approach, (B) dimensions of the microparticle system simulated mimicking the experimental design with a 4X core, (C) boundary conditions in the simulations, (D) meshed geometry of the numerical model, (E) time snapshot of microparticle deformation, (F) final modes of deformation for different numerical conditions (noted by E) from the DOE table in nonporous model, and (G) two dominant modes of deformation observed in the porous model. Effect of the numerical parameters on normalized vertical displacement in the cap in (H) nonporous solid mechanics model and (I) poroelastic model. (J) Experimental vertical displacement of the cap and comparison with cumulative in vitro release of AF647-labeled dextran from the core (n = 4). Release profile depicted here is averaged among four cargo molecular sizes studied in Fig. 3A. (K) Normalized vertical displacement of the cap as a function of cap porosity based on simulations. Error bars in (J) show the standard error of the mean. Photo credit: Morteza Sarmadi, Massachusetts Institute of Technology.
patterns, labeled as shrinkage ($V_{\text{mean}} < 0$) or expansion ($V_{\text{mean}} > 0$) (Fig. 5G). Next, the effect of the four dimensionless parameters on $V_{\text{mean}}$ was investigated. Simulations revealed that nonporoelastic and poroelastic models displayed different deformation behaviors (Fig. 5, H and I). In the nonporoelastic model, $R_p$ was found as the most important factor, considerably influencing the deformation of the cap, particularly going from $R_p$ of 0.1 to 0.2 (Fig. 5H). In this study, to capture a broader range of conditions for the cargo, $R_p$ varies, meaning that the osmotic pressure inside and outside can be variable. The actual osmotic pressure inside the core is dependent on the type (electric charge) and the amount of cargo (molarity) loaded in the core and varies among different cargos. Simulated cap displacement reached a plateau at $R_p$ of 5 and $R_p$ of 0.1 in the nonporous model. These results indicated that microenvironment pressure ($P$), captured by the change in elastic modulus ($E$), instead of polymer hydrolytic degradation, can be the dominating factor controlling the deformation of nonporous particles. However, in the poroelastic model, the effect of the parameters depended on the porosity ratio ($R_h$) (Fig. 5I). Unlike the nonporoelastic model, in the poroelastic model, $R_E$ was found as the dominant factor among all porosity values studied. This could suggest that, in a pore forming model, the change in the material properties of cap and base, captured by $R_E$, dominates the release time point. Increasing parameter $R_p$ decreased $V_{\text{mean}}$, suggesting that increased hydrostatic pressure relative to the core pressure reduces cap deformation.

As the next step, we calculated the experimental equivalent of the vertical displacement of the cap, $V_{\text{mean}}$ (normalized by mean; Fig. 5J), in cross-sectional SEM images of microparticles over time ($n = 4$). An image processing approach was followed to model the cap as a deformed rectangle. Full description of the protocol for the calculation of experimental $V_{\text{mean}}$ can be found in the Supplementary Materials. We observed that vertical deformation of the cap consisted of three phases: (i) an initial expansion (up to day 4), (ii) followed by a shrinkage (days 4 to 8), and, lastly, (iii) a sudden expansion (days 8 to 10). We then compared cap displacement with the corresponding data on release kinetics, averaged across all cargo sizes studied in Fig. 3 for a 4X-core, 100-μm cap particle design (Fig. 5J). It was observed that the secondary jump in the average vertical displacement of the cap (third phase in cap deformation) corresponded to the onset of pulsatile release.

Among 18 different simulated conditions, provided by the DOE (an $L_{18}$ orthogonal array), condition 5 was found to be the closest to capture a trend of deformations similar to experimental results (Table S1). The trend of numerical $V_{\text{mean}}$ as a function of cap porosity in this model followed a qualitatively similar pattern to change in the experimental $V_{\text{mean}}$ as a function of time, capturing cap degradation upon incubation. The criteria and assumptions for comparing simulations and experimental findings are presented in the Supplementary Materials (see the “Cap vertical displacement calculation” section). We found a numerical porosity threshold (22.5%, core excluded) that led to a sudden jump (more than sixfold) in $V_{\text{mean}}$, which is in agreement with experimental observations. While the initial expansion/shrinkage phase observed in the experiments was captured with a lower peak in the numerical model (core excluded), the overall trend of the three phases was consistent with the numerical model (Fig. 5K).

**DISCUSSION**

In this work, various tools from experimental and computational approaches were integrated to study the complex mechanism of degradation in biodegradable core-shell microparticles. A comprehensive study was conducted to investigate key factors controlling the mechanism of pulsatile release. It was demonstrated that pore formation and deformation in the microparticle cap were the two governing parameters that control the release kinetics. Since the release profile is independent of particle geometry within the range studied (100- to 500-μm particles), this approach can be applicable to a wide range of core-shell particle designs. The framework presented here can also be used to systematically study the complex degradation of future biodegradable materials.

PLGA microspheres and their degradation have been studied over the past three decades (20, 22). Although widely investigated, the underlying mechanism and functional impact of PLGA degradation are often dependent on the specific microstructure of the drug delivery system. In the system described in this work, core-shell microparticles, we observed an erosion-like behavior that deviated from degradation of emulsion-fabricated PLGA microspheres with preexisting pores. PLGA has been widely recognized as a hydrolytically bulk degradable polymer in which pores are expected to develop throughout the entire polymer matrix upon hydrolysis (20–24). For the core-shell particles studied here, pore formation was more pronounced on the edge of the cap due to higher interfacial surface area with water, leading to a regional degradation front on the edge of the particles. The locally degraded front eventually progressed throughout the entire particle (or particle cap), reaching the core, but rather slowly in order of days.

The pore formation followed a nonlinear trend, with porosity being similar on days 3 and 7 while drastically increasing on day 10 (Fig. 1) for a particle with in vitro release at day 8 ± 1. Interconnected pores led to the formation of a path connecting the cargo to the outer environment, leading to pulsatile release (Figs. 1 and 2). This pronounced degradation pattern near release time point was further supported by results from thermal/mechanical analysis, showing a sudden drop in $T_g$, $T_d$, and $E$ within 8 to 10 days (particularly at day 10). Formation of a tortuous network of pores within the PLGA matrix, facilitating release of drugs to the medium, has been postulated in the literature (22, 25). Some studies also experimentally visualized the hydrolysis-caused formation of interconnected pores across cross sections of PLGA microspheres but without demonstrating release of the drug itself (17, 24). In contrast, this study demonstrates that pore network formation in PLGA can be used as a “gate” to enable the pulsatile release of a macromolecular cargo from the core of PLGA microparticles. We provide direct visual evidence of release of a model macromolecule (10-kDa dextran) through the bulk of the PLGA matrix using EDS-SEM and OM (Fig. 2).

This study further suggests that particle composition, especially polymer MW and end group, and not size or morphology, dominates the release time point in core-shell microparticles within the size range studied (~100 to 500 μm). Uniquely, the delayed pulsatile release profile was preserved across all 25 different designs of microparticles studied (Fig. 3). Size independence of release time point from particle geometry (within ±2 days in vitro), within the range studied, provides an advantage over existing drug delivery systems such as hydrogels and microspheres (26, 27). Accordingly, as long as the composition of the cap stays the same, particles with different morphology can essentially provide predictable release kinetics. This could potentially allow for the physical design of the core-shell particles to be tailored to specific administration methods or therapeutic applications where a certain particle size or morphology is required.
It is critical to investigate whether particle deformation patterns observed in the sample size studied ($n = 3$ to $5$) can be expanded to a larger population size. First, the microfabricated particles have previously shown to be monodisperse and have a predictable pulsatile release (1, 2). Moreover, we calculated the projected surface area of the particles for all groups (80 different groups, each group with $n = 3$ to $5$ particles, $\sim 400$ particles) based on SEM, OM, and nanoCT imaging (data file S1). We analyzed the confidence interval (CI) for 95% of confidence level among all images. The percentage of CI for each group was then calculated as $CI = CI/100$/average. The CI percentage was found to be $13.69 \pm 12.85\%$ ($n = 80$), showing an acceptable level of difference among particles in terms of deformations. The data file reporting all analysis and CI percentages can be found in the Supplementary Materials (data file S1).

This study qualitatively assessed microclimate pH change, a key factor affecting release, in the cap and base of core-shell microparticles (Fig. 4). Various methods have been developed to quantify pH distribution in PLGA microspheres such as laser scanning confocal microscopy or two-photon microscopy (28, 29). However, these techniques suffer from limitations on the detectable acid range (pH $\sim 2.0$) and complexity of the protocol (28, 29). The method presented here, based on incorporation of TB, is a simple qualitative technique that covers a lower acidic range ($\leq 1.2$). It also provides visual understanding of pH distribution within relatively large (>100-μm) PLGA-based systems.

The biphasic map of pH, representing higher pH on the edge compared to the center, has also been reported in PLGA microspheres (19). The higher pH region on the edge, progressing to the center, can be attributed to diffusion-mediated transport of acidic products to the environment (19). Over time, as the cap surface porosity increased, the portion of the less acidic front (yellow) increased until it covered the entire cap (Fig. 4). Further comparison between sealed cap and cap-only microparticles also revealed that acidic PLGA degradation by-products may accumulate in the core and center of the cap over time, coinciding with cap expansion. The progressive swelling facilitated by pore formation in the cap further contributes to pulsatile release of the cargo potentially at a threshold porosity.

The association between PLGA swelling and sudden release of the cargo has also been observed in PLGA microspheres (18).

Given the pore-mediated release mechanism, we then postulated that addition of PEG, a widely used pore-forming agent, can potentially alter the release kinetics (28, 30, 31). We observed that blending PEG as an additive into the cap resulted in earlier release compared to pure PLGA, while blending PEG-PLGA block copolymer with PLGA had no effect on the release time. PEG, especially at the MW used (2 kDa), when directly blended with PLGA, serves as a porogen (30, 31). Upon interacting with water molecules, PEG dissolves and leaves the PLGA matrix, creating voids within the cap, as shown by surface SEM (Fig. 4). In addition, the presence of these voids within the matrix creates larger water pathways through the particles that, combined with higher hydrophilicity, leads to enhanced water uptake and hence faster hydrolysis of PLGA. This enhanced water intake and earlier release point also coincide with higher pH (pale yellow) in PEG-doped PLGA particle groups compared with pure PLGA. Pronounced pore formation can enhance clearance of acidic products out to the buffer by increased water permeation (Fig. 4). Conversely, when PEG is tethered to PLGA (PEG-PLGA), the degradation would be dominated by swelling and higher water uptake rather than pore formation. Swelling of PEG segment at 2 kDa, as used here, is likely not high enough to change the release time. The PEG domains in PEG-PLGA are protected against water interactions due to increased protection by the hydrophobicity of PLGA.

To study the effect of parameters not captured in the experiments, a Multiphysics numerical model was developed. Advanced computational tools are becoming increasingly important in studying biomedical systems in recent years (32–35). To our knowledge, this is the first study to use the theory of poroelasticity to numerically model deformation in a polymeric biomaterial. Studying the parameters in a dimensionless form enables broader insights into the behavior of the system. The DOE approach also has been successfully used for studying complex engineering systems (35–38). This approach helps optimize the number of required experiments to systematically study the model. The numerical results in the porosity domain matched the trend of experiments in the time domain. They both pointed to the presence of three modes of cap deformation: (i) an early expansion, followed by subsequent (ii) shrinkage, and, lastly, (iii) a sudden expansion attributed to the onset of pulsatile release (Fig. 5). We also observed that decreasing the ratio of elastic modulus of the cap relative to the base (increasing $R_E$) leads to a sudden expansion of the cap, similar to the experimental results. The peak cap expansion was observed taking place at a threshold $R_E$, dependent on the porosity of the microparticle. In this study, with the assumption that the elastic module of the base remains always greater than that of the cap, it can be deduced that increasing $R_E$ captures the decrease in the elastic modulus of the cap upon hydrolysis. Accordingly, an increase in $R_E$ can capture increased degradation of the particle cap over time. This agrees with numerical results derived from increasing porosity, indicating a certain threshold for $R_E$ after which the cap experiences a sudden expansion.

Numerical results also demonstrated that increasing $R_P$ led to pronounced expansion of the cap (for $R_P > 1$). As shown, sudden expansion of the cap was concomitant with the release of cargo, as also reported in PLGA microspheres (18). Therefore, cargos for which core pressure outweighs hydrostatic pressure ($R_P > 1$) are expected to increase cap expansion. Water permeation into the core before the release time point can also contribute to additional pressure in the core, generating a pressure gradient from the core toward the buffer. This pressure gradient can be another cause of cap expansion. As higher-MW PLGAs resist water permeation, they are expected to release the cargo at a later time point, as experimentally demonstrated (Fig. 3). The current multiphysics model can be used to provide a roadmap toward modeling deformation of PLGA-based drug delivery systems in future studies.

Collectively, the mechanism of pulsatile release in biodegradable core-shell microparticles can be triggered by hydrolysis of PLGA molecular chains (Fig. 1A). The hydrolysis reactions lead to cleavage of PLGA molecular chains, captured by a decrease in $T_p$ and $E$ of the particle cap (Fig. 1G and figs. S4 and S5). Cleavage of PLGA molecular chains over time also leads to pore formation (Fig. 1) and accumulation of acidic products (Fig. 4). All these factors then contribute to cap deformation through intermediate processes such as a pressure differential or poroelasticity of the cap (Fig. 5). Cap deformation (expansion or shrinkage) and pore formation are two key factors that eventually lead to pulsatile release from core-shell particles at a threshold porosity/expansion ratio. The pore formation upon PLGA degradation has already been reported in the literature. The unique aspect of this study is investigating the manufacturing technique that enables fabrication of particles in a core-shell hollow microstructure, potentially expandable to other polyesters.
Overall, this study aimed to use various experimental and computational tools to study the mechanism of pulsatile release in biodegradable core-shell microparticles. To the best of our knowledge, the current work is the first to integrate multiple tools from experimental and computational tools to study pulsatile release kinetics from biodegradable microparticles. Results of this study not only can provide insights into the mechanism of pulsatile release but also can enhance general understanding of degradable biomaterials such as Poly(L-lactide), (PLLA). Furthermore, they can aid in the design and fabrication of next-generation biomaterials and drug delivery systems for a broad range of drug delivery applications including self-boosting vaccines.

**MATERIALS AND METHODS**

**Fabrication of biodegradable core-shell microparticles**

**SEAL method**

Core-shell microparticles were fabricated using a slightly modified version of the recently developed multilayer microfabrication technique termed SEAL (1). Full description of the fabrication process can be found in (1, 2). Briefly, positive master molds for caps and bases were fabricated using SU-8 lithography, made by Front Range Photomask (AZ, USA). Films of pure PLGA or mixed with other additives were pressed into negative molds made from polydimethylsiloxane (PDMS) (Sigma-Aldrich) for particle caps and bases. An additional Teflon layer was used to cover the film in PDMS and retain PLGA in the molds for the cap arrays. The bases contained a cubic hollow cavity (core) that was filled in multiple cycles with the cargos of interest by high-precision piezoelctric picoliter dispensers, namely, BioJet Ultra picoliter dispensing instrument (BioDot, CA, USA) for low-viscosity cargos and cellenONE (Scienion AG, Berlin) for high-viscosity cargos. Filled array of bases on a glass slide was then aligned and sintered with the corresponding PLGA caps under a microscope using a photomask aligner (MA4, Karl SUSS) or a custom-made, laboratory-developed mask aligner device both equipped with a Peltier heater for simultaneous alignment and sealing. The resulting core-shell microparticles were cut and removed using tweezers and a razor blade. Various PLGA powders were purchased from Evonik and PolySciTech. Microparticles in this study were fabricated from different cap and base PLGA Resomers. Details of particle design and the amount of cargo loaded into the core in various experiments can be found in data file S1. Unless otherwise stated, the base layer was made from high-MW PLGA Resomer RG 756S. Microparticles with blended caps studied in Fig. 3F were fabricated using films made from a mixture of different PLGAs blended at a given ratio (dissolved in acetone). Microparticles studied in Fig. 3D were fabricated by nanoresolution 3D printing (NanoScribe) at the Center for Nanosystems, Harvard University, as described in the following.

**NanoScribe additive manufacturing (NanoScribe)**

**Printing process.** Particle geometries were designed using SketchUp and DeScribe software. Prints were made on a Photonic Professional GT NanoScribe 3D Lithography system using the DiLL method with IP-S photoresist. Prints were developed in propylene glycol methyl ether acetate and isopropanol according to the manufacturer’s instructions and dried for 30 min on a hot plate at 90°C.

**PDMS mold fabrication.** NanoScribe prints were coated with silicone in a desiccator for 30 min before molding. PDMS was prepared with a 7.3:1 ratio of base to cross-linker and centrifuged for 15 min at 5000 rcf. The PDMS was poured over the mold and degassed under vacuum for 60 min. This was cured overnight at 60°C. Some molds were found adequately rigid after these steps; however, in some cases, additional processing was necessary and described as follows.

The preliminary PDMS mold was again coated with silane under vacuum for 30 min. The mold was filled with NoA Optical Adhesive 61 (Norland Products, Cranbury, NJ, USA). This was degassed under vacuum for 60 min, followed by ultraviolet (UV) curing for 30 min. The UV mold was then baked at 120°C for an additional 60 min to prevent off-gassing of any residual adhesive that could disrupt the PDMS molding process. Further working PDMS molds were constructed off the UV master using the above PDMS mold protocol.

**Microparticle imaging and image analysis**

SEM and EDS-SEM were performed using a JSM-5600LV SEM (JEOL, Tokyo, Japan) with an acceleration voltage of 5 to 10 kV. Samples were initially coated with a thin layer of Au/Pd using a Hummer 6.2 sputtering system (Anatech, Battle Creek, MI, USA) and then imaged. EDS was performed in the dark field, and both SEM and EDS-SEM were performed at high vacuum settings. Samples for cross-sectional analysis were first frozen by liquid nitrogen and then cut under an optical microscope. Iron(II) sulfate heptahydrate (FeSO₄·7H₂O) was purchased from Sigma-Aldrich and dispensed (100 ng) into 4X-core particles using a cellenONE picoliter dispenser. Nanoresolution x-ray CT reconstructions (resolution, 0.38 to 0.45 μm) were obtained at the Biotechnology Resource Center of Cornell University. NanoCT imaging was performed using a ZEISS/Xradia Versa 520 x-ray microscope. The three cross sections depicted in Fig. 1 for each particle were selected to be equidistant (50 to 60 μm apart). Reconstruction of nanoCT scans was performed with Avizo software (Thermo Fisher Scientific, USA). All 2D image analysis including porosity measurements was performed by ImageJ.

**Release kinetics measurement**

In all release measurements in this study, an individual particle was placed into 125 μl of PBS in a low-bind microcentrifuge tube (Eppendorf, Hamburg, Germany) and incubated on a shaker (100 rpm) at 37°C. Before starting the experiments, tubes were microcentrifuged to make sure that particles completely sink inside the buffer in the tubes. All release measurements were performed daily for at least six particles per group (mostly 10), by analyzing the supernatant fluorescence using a Tecan Infinite M200 spectrophotometer (Männedorf, Switzerland). The excitation/emission wavelengths of 650 nm/680 nm were used for all AF647-labeled dye detection. Results were normalized to the total cumulative release. For each measurement, 100 μl of supernatant was taken from the tube (after gently resuspending the buffer multiple times). Supernatant was replaced with 125 μl of fresh PBS after each measurement. Release of four different types of AF647-labeled fluorescent dyes loaded in the core was analyzed. Dextran-free and 10-kDa dextran groups were purchased (Thermo Fisher Scientific, Waltham, MA, USA). Groups of 70 and 2000 kDa were manually labeled with AF647. Briefly, dextran was dissolved at 10 mg/ml in a pH 8 buffer and then added 10-fold relative to calculated amines in N-hydroxysuccinimide–conjugated AF647 dye. The resulting mixture was left to react for 2 to 4 hours at room temperature and then dialyzed with a cassette. The resulting mixture was spin-filtered using Zeba Spin Desalting Columns.
Fabrication of microparticles with PEG-loaded cap
PEG was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). A certain amount of PEG (pure or PEG-b-PLGA) was added to acetone/PLGA mixture. In case of pure PEG, 50 or 100 mg of 2-kDa PEG was mixed with 500 mg of PLGA and subsequently added to acetone. The resulting PEG/PLGA/acetone mixture was magnetically stirred, sonicated, and slightly heated up (up to 40°C for 30 min) until a homogeneous solution (in acetone) was achieved. Films made from PEG/PLGA were fabricated by first pipetting the solution on a Teflon-coated glass slide and then sliding the slide under a doctor’s blade, followed by overnight drying on hot plate at 45°C. The resulting films had an average thickness of 40 to 70 μm. PEG-b-PLGA–loaded caps were fabricated similarly except that PEG-b-PLGA (50 or 250 mg) was used instead of pure PLGA. The resulting films were used to fabricate the microparticle caps using micromolding, as previously described.

Encapsulation of pH indicators
Universal pH indicator [300 ng pH indicator solution (pH 4.0 to 10.0); Sigma-Aldrich Inc.] was dispersed (at a concentration of 1 mg/ml) into the core of microparticles by a high-precision picoliter dispenser device, BioDot. TB (20 mg; Sigma-Aldrich, Inc.) was added to 1 ml of the PLGA (50%, w/v) in acetone solution film precursor. The resulting mixture was sonicated for 1 hour and then used to fabricate polymer films and, subsequently, microparticle caps, according to the process previously described.

Numerical model in COMSOL and design of numerical simulations
A total of 52 simulations were performed (18 based on numerical DOE for four different porosity ratios). All simulations in this study were conducted using COMSOL Multiphysics V5.2 (Burlington, MA, USA). Solid mechanics module and poroelasticity module were coupled during simulations. Accordingly, the solution that resulted from simulation of the solid mechanics model was the input to the porous model as the initial condition. The numerical DOE was designed and analyzed by Minitab software (Chicago, USA) based on a L18 Taguchi orthogonal array repeated for various levels of cap/base porosity ratios. Table S1 summarizes the 18 conditions studied. The underlying mathematical formulation for simulations can be found in the Supplementary Materials.

Thermal analysis
DSC analysis was performed using the PerkinElmer DSC 8000 System under nitrogen flow (50 ml/min). For DSC analysis, a heating-cooling-Heating cycle was applied from −80°C to 80°C, with heating and cooling rate set at 10°C/min to determine the glass transition temperature (T_g) temperature of the tested polymer blends. Furthermore, TGA was conducted in a Pyris 1 Thermogravimetric analyzer with heating rate at 20°C/min from 50° to 600°C under nitrogen flow (20 ml/min). Degradation temperatures (T_d) of tested polymer samples were determined at the maximum rate of weight loss. All sample analyses were performed in at least triplicate. Thermal analysis was performed on PLGA 502H films submerged in PBS, incubated at 37°C on a shaking incubator (100 rpm), and sampled at different time points.

Mechanical compression analysis
Samples were subjected to compression testing using a material testing machine (Instron 5943, Norwood, MA, USA). Samples were set up such that a single microparticle was underneath the compression clamp. To evaluate elastic modulus, two extension rates were used: 0.09 mm/min (days 0 to 8) and 0.015 mm/min (days 9 and 10). Elastic modulus was defined by measuring the slope of the linear portion of the curve immediately before maximum load. Mechanical tests were performed on a cubic solid (without core) microparticle (350 μm by 350 μm by 320 μm) made from the fast-degrading PLGA (RG 502H), incubated at 37°C in PBS (shaking incubator, 100 rpm), and sampled at different time intervals, to model degradation of the cap in core-shell particles.

Statistical analysis
Statistical test between two groups was performed on the basis of Student’s t test. A P value of less than 0.05 was considered statistically significant. Statistical analyses were performed in GraphPad Prism (GraphPad Software, La Jolla, CA).

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at https://science.sciencemag.org/content/sciadv/8/5/eabn5315.suppl.

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Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

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