Micromolding of Thermoplastic Polymers for Direct Fabrication of Discrete, Multilayered Microparticles

Ilin Sadeghi, Xueguang Lu, Morteza Sarmadi, Robert Langer, and Ana Jaklenec*

Soft lithography provides a convenient and effective method for the fabrication of microdevices with uniform size and shape. However, formation of an embossed, connective film as opposed to discrete features has been an enduring shortcoming associated with soft lithography. Removing this residual layer requires additional postprocessing steps that are often incompatible with organic materials. This limits adaptation and widespread realization of soft lithography for broader applications particularly in drug discovery and drug delivery fields. A novel and versatile approach is demonstrated that enables fabrication of discrete, multilayered, fillable, and harvestable microparticles directly from any thermoplastic polymer, even at very high molecular weights. The approach, isolated microparticle replication via surface-segregating polymer blend mold, utilizes a random copolymer additive, designed with a highly fluorinated segment that, when blended with the mold’s matrix, spontaneously orients to the surface conferring an extremely low surface energy and nonwetting properties to the template. The extremely nonwetting properties of the mold are further utilized to load soluble biologics directly into the built-in microwells in a rapid and efficient manner using an innovative screen-printing approach. It is believed that this approach holds promise for fabrication of large-array, 3D, complex microstructures, and is a significant step toward clinical translation of microfabrication technologies.

1. Introduction

3D microdevices with uniform size and shape are of high interest for a wide range of fundamental applications from electronics, optics, and energy to biomedicine. Extending the breadth of microfabrication techniques, originally developed for microelectronics, to the biomedical arena has led to significant advancement in diagnostic and therapeutic modalities. Soft lithography, microtransfer molding (μTM) in particular, is especially attractive due to its simplicity, ability to fabricate structures with controlled size and microarchitecture over a relatively large area and compatibility with organic materials. μTM involves using an elastomeric mold, most commonly poly(dimethylsiloxane), PDMS, due to its low surface energy, “high flexibility, optical transparency, and low volumetric shrinkage” allowing for conformal contacting and high fidelity pattern transfer. In this method, a polymer is pressed against a PDMS mold and heated to above its glass transition temperature ($T_g$) to produce the polymeric replicas of interest. However, microstructures formed on the substrate are all connected through a residual film called scum layer (Figure 1A), meaning for their application, the microparticles need to be precisely removed from the embossed film. Formation of this connective film has been an inherent shortcoming of μTM technique from its early development stage to its current state, and has limited its scalability and translation for applications in diverse fields particularly in drug delivery.

Several approaches have been utilized to address this problem and create isolated particles, from a simple mechanical force or mechanical punching to harsher approaches such as reactive ion etching (RIE) or laser cutting. Collectively, these methods not only add a postprocessing step, but more importantly, they can compromise the particle integrity and/or cargo viability specially for sensitive cargos. For example, RIE which is perhaps the most common method for removing the residual layer, integrates well with rigid, inorganic materials for microelectronics, but its compatibility with polymers, relevant for biomedical applications, is highly limited especially when particles contain sensitive drug/biologics. The process can form complex by-products with concerning toxicity profiles or excess heat that can be damaging to the cargo. Similarly,
applying excess pressure results in the distortion of the mold and deformation of the patterns.\textsuperscript{[8]} Another example is utilizing a PDMS microwell stamp dip-coated with a thin polymer film, and polymer on the ridges between the microwells is then removed by microcontact hot printing.\textsuperscript{[12]} Next, the stamp is pressed against a water-soluble release layer to transfer the features formed in the recessed area of the mold. Since pressure is required to release the features from microwells, only a subset of microfeatures size and shape (i.e., very low aspect ratio features) can be made. It is also subject to formation of nonuniform features as a result of partial film coverage by the dilute coating solution.\textsuperscript{[13]} Alternatively, a phase reversible dissolvable hydrogel template is utilized to achieve discrete microstructures.\textsuperscript{[14]} After solvent evaporation, the formed particles are released upon dissolving the mold in water at 40 °C. However, nonuniform solvent evaporation and lateral contraction during drying are reported to result in particle size dispersity, and concavity in the center of the features.\textsuperscript{[13]} Additionally, hydrogel templates are not mechanically strong enough to withstand pressure for pattern transfer onto a substrate, requiring dissolution of template in warm water to release the particles. This could adversely affect particle integrity and cargo viability. Moreover, particles released in water are not suitable for fabrication of multilayered structures where further alignment of the features is required. Particle replication in nonwetting templates (PRINT) is likely the most versatile and well-controlled method for direct replication of isolated, shape-specific nano/microstructures (<5 μm) using a nonwetting crosslinked perfluoropolyether (PFPE) mold.\textsuperscript{[16]} While this is an elegant method for its nanoscale resolution, it relies on in situ polymerization/crosslinking that may not be compatible with the encapsulation of sensitive drugs or other fragile biologics due to presence of precursors (e.g., catalyst, photoinitiator, monomer, and solvent) that could denature or solubilize the payload. It would also require purification of the formed particles from any unreacted reagents and any remaining impurities that might be too harsh for encapsulated cargo, especially for sensitive biologics. Additionally, the PRINT-fabricated particles are limited to single-layer geometries that can be released from the mold, making it not suitable for fabrication of structures with internal architecture or top-narrowing features. Recent advances in soft lithography and development of new methods such as configurable, elastic crack engineering (CECE) has widened the scope of soft lithography for replication of exquisite 3D hierarchical structures consisting of closed-loop regions of diversified materials, originally only possible with high-resolution 2-photon 3D printing of rigid materials (e.g., polystyrene) not suitable for biomedical applications. However, the solid features are still formed on a backing film required for complete release from the mold. The method also still falls short in creating features from high viscosity polymers due to their limited fluidity to fill the cavity.

Figure 1. A) Traditional μTM process using PDMS mold. B) IMPRESS technique, in which the extremely nonwetting properties of the templates, emanating from addition of a new surface segregating copolymer additive, enables direct fabrication of discrete microparticles from high molecular weight polymers.
Compared to liquid monomers, generating isolated microparticles directly from polymers is even more challenging due to significantly higher viscosity of polymer melts than corresponding monomers. In-situ polymerization of low viscosity monomer solution provides high flowability and redistribution within the mold cavities for full patterning.\[21\] In comparison, direct fabrication of particles from polymers involves heating the polymer to above its $T_g$, at which it behaves as a viscous liquid and starts “flowing” and thereby conforming to the mold.\[19\] Highly entangled polymer chains and intermolecular forces between them at their melt state, inherent to polymers, increase their resistance to flow.\[18,20\] The higher the polymer molecular weight (MW), the lower is its flowability, reflected by low polymer melting flow index.\[21\] Moreover, the filling of the micron-size cavities of the mold is further slowed down since free flow is restricted.\[22\] The viscoelastic properties and low fluidity make it challenging to selectively fill only the recessed area of the mold leading to formation of an even thicker embossed layer adhering to the substrate and interconnecting the features, compared to the low viscosity precursors.

Hence, lack of a versatile and practical technique capable of generating discrete features of high fidelity from a wide range of commercially available biocompatible polymers in a cost-effective and high-throughput manner sets limit on expanding the microfabrication realm to drug delivery applications. The challenge is especially acute when fabrication of 3D, multilayered microfeatures is of interest, as this means scum layers stack upon addition of each layer and further interconnect the individual features. New approaches that enable creation of discrete, 3D, multilayered features with internal architectures directly from high viscosity polymers can significantly expand the breadth of microfabrication technique. Such microstructures, if developed precisely and reproducibly via an easy-to-implement method, could potentially surmount the challenges associated with conventional delivery devices and find new capabilities including delivery of sensitive biologics, improving compliance or localizing the cargo delivery.\[22\] This would be a significant leap toward development of more efficient drug delivery systems and has implications in a myriad of biomedical fields including vaccinology, immunotherapy, and hormone therapy.\[24,25\]

Towards the goal of addressing these challenges, we have developed a simple, single step, yet highly versatile platform, that enables direct fabrication of discrete, uniform, multilayered particles from a variety of biocompatible, commercially available polymers with high resolution even at a very high MW. This approach, termed isolated microparticle replication via surface-segregating polymer blend mold (IMPRESS) draws on elements from well-established technologies including soft lithography and thermal lamination and combines them with engineered surface chemistry to create an extremely nonwetting template for replication of discrete biocompatible microdevices with defined architecture. IMPRESS involves the design of a highly fluorinated, surface-active random copolymer additive (FRCP), poly(dimethylsiloxane-random-perfluorodecyl acrylate), P(DMS-\(\tau\)-PFDA), containing a PDMS segment and a fluorinated segment (Figure 1B), extensively reported in the literature for its nonwetting properties in a wide range of applications.\[26,27\] The additive is blended with the bulk PDMS and curing agent during fabrication step, rendering it readily adaptable to classical soft lithography technique with no additional postprocessing step. To enmesh the additive within the PDMS bulk matrix for its retention upon repeated use, the copolymer is designed with a PDMS segment. This ensures high miscibility with the PDMS base elastomer and serves to “solubilize” the additive within the bulk matrix during preparation, and later provides strong anchoring sites to the PDMS matrix.\[26\] Driven by their low surface energy, long, fluorinated side chains of PFFDA segregate to the polymer/air interface, a thermodynamically favorable state, turning the surface extremely nonwetting to organic materials.\[27,29\] The fluorinated mold, termed Fold, developed here, features a surface composed of $-CF_3$ groups with the lowest surface energy attainable (6 dyn cm\(^{-1}\))\[30\] compared to that of PDMS (21.6 dyn cm\(^{-1}\))\[31\] or Teflon (18.5 dyn cm\(^{-1}\))\[32\] with $-CF_2$ groups similar to that of PRINT template. The substrate onto which particles are transferred to is also coated with a thin layer of the same material by spin-coating a solution of PDMS blended with FRCP, and subsequently cured at a similar condition as the Fold, turning both the Fold, and the substrate nonwetting to the organic materials to be molded. Upon clamping the Fold against the substrate and subsequent heating, the polymer fills the shaped cavities within the Fold and the excess polymer is forced out due to the extremely low surface energy of the template. The low adhesive forces allow for easy release of the Fold from the features with minimal mechanical force, leaving isolated features with high fidelity replication on the substrate. Thus, the collective properties of high flexibility of the pendant side chains to preferentially orient to the surface as well as their extremely low surface energy imparts superb nonwetting properties to the templates, outperforming the PFPE molds (composed of $CF_2$ groups within the main polymer chain). The mobility of pendant chains and its preferential orientation to the surface is especially favored when the templates are annealed above the $T_g$ of the PFDA segment ($\approx 50$ °C)\[33\] leading to even higher surface fluorene enrichment at the condition in which microstructures are molded. The simple, blending approach used in this study is not only advantageous in cost, but more importantly, allows tailoring the surface chemistry independently from the bulk properties.\[27\] Thus, the excellent optical and mechanical properties of PDMS remained unaltered while the surface properties can be tuned to achieve low adhesion and promote Fold/substrate release for various geometries. Using IMPRESS technique, we generated topologically complex, multilayered microdevices with high fidelity in a simple and reproducible manner. This scalable technique to generate 3D microparticles with extensive flexibility in engineering microparticles size and morphology could potentially enable fabrication of new and complex drug delivery devices and offer opportunities to further illuminate the role of physicochemical properties of such devices for a more advanced design.

Here, we designed a new copolymer additive, P(DMS-\(\tau\)-PFDA) with varying monomer ratios (15/25 wt% PFDA) that imparts the desirable nonwetting character to the molds when blended with PDMS. The copolymer was synthesized using free radical polymerization, a simple, robust, and scalable synthesis method.\[14\] Detailed synthesis procedure and copolymers’ characterization are explained in Experimental Section. Next, Folds
were fabricated following a similar procedure commonly used for fabrication of PDMS molds (Experimental Section) except for the added FRCP to the PDMS precursor. Folds showed similar optical transparency to the unmodified PDMS molds (even at a concentration up to 2 wt%), suggesting the high miscibility of the additive within the PDMS bulk matrix,[35] compared with whitish molds obtained when commercially available block copolymer additive, poly(dimethylsiloxane-block-tridecafluorooctylmethylsiloxane), (P(DMS-b-TDFOMS), Gelest), was used even at a very low concentration. The close proximity of short segments of random copolymer architecture could have likely contributed to well integration of the additive within the bulk matrix. The optical clarity of the mold is an essential property required for fabrication of complex multilayered geometries as well as other potential applications of the technique including microfluidics.

To demonstrate the robustness and broad material compatibility of IMPRESS technique, shape-specific organic particles composed of three different biomedically-relevant thermoplastic polymers were generated (Figure 2). As the first demonstration of the utility of this method, poly(lactic-co-glycolic acid), PLGA (141 kDa and 91 kDa) rectangular cubic microparticles were fabricated. PLGA has been the most widely explored class of synthetic polymers for drug delivery applications due to its biocompatibility, safe toxicity profile, minimal foreign body reaction and commercial availability at varying MWs.[36] It has a relatively low $T_g$ (40–60 °C depending on MW and PLA:PGA block ratio),[36] which makes it a suitable material to be molded. To fabricate particles using standard soft lithography, a PLGA film was pressed against PDMS mold and was then transferred to a vacuum oven at 120 °C for about 7 h. After pattern transfer was completed, the mold and the glass were separated, leaving an array of microstructures with an embossed film throughout the whole particle array on the glass (Figure 2A). To further examine the resolution and replication quality of the features, they were separated from the glass using a razor blade and were transferred onto a carbon tape for SEM imaging. As represented in Figure 2A, and Figure S2A,B (Supporting Information), the thick scum layer makes it impossible to separate a single particle from the connective film without leaving residues all around the particles. IMPRESS uses a similar procedure to generate particles but in a non-wetting template in which PLGA film is clamped against the Fold and nonwetting substrate, prepared by spin-coating of a solution containing PDMS, curing agent, and FRCP additive at the desired concentration on a flat glass surface and cured at the same condition as the Fold. Upon delamination onto the coated glass, uniform, discrete particles were formed and were then easily transferred by a razor blade for SEM imaging (Figure 2B). Moreover, higher magnification images (Figure S2C,D, Supporting Information) further illustrate a single particle with sharp edge definition and no residue attached, clearly demonstrating the high fidelity of the method. Of note is that the formed features were easily delaminated from the Fold; however, achieving high-quality

![Figure 2.](image)

**Figure 2.** A–D) SEM images of shape-specific particles fabricated from various polymers using A) PDMS mold, B–D) Fold. E–H) PLGA multilayered microstructures; sealed core–shell PLGA (141 kDa) particles with size of 400 × 400 × 300 μm generated by E) PDMS mold F–L) Fold G) sealed core–shell cubic PLGA (19 kDa) microparticles with size of 160 × 160 × 160 μm; H) letters spelling “MIT” and curved flag-like PLGA (141 kDa) microstructures. I–L) Optical images of PLGA bases (141 kDa) at varying sizes I) 160 × 160 × 130 μm particles with 30 μm wall thickness, J) 400 × 400 × 250 μm particles with 50 μm wall thickness, K) Filled and sealed 400 × 400 × 200 μm particles with 100 μm wall thickness, L) 400 × 400 × 200 μm particles with 100 μm wall thickness harvested on tip of a razor blade.
micropatterns using PDMS mold required an additional surface modification step (i.e., salinization by vapor deposition of a fluoroalkyl trichlorosilane) to alter surface properties and promote the release for a full pattern transfer.

To further examine the versatility of IMPRESS technique, we selected another class of synthetic, biocompatible polymer for its considerable impact on biomedicine and drug delivery; poly(methyl methacrylate) (PMMA) and derivatives thereof (methacrylate copolymers) are widely explored as carrier materials due to their chemical properties and toxicological safety record in humans.\(^{[17]}\) PMMA derivatives are especially promising as encapsulant materials for oral delivery of sensitive biologics and nutrients with low bioavailability, offering protection from the harsh gastrointestinal environment.\(^{[18]}\) As an example of the PMMA-based bioavailability, offering protection from the harsh gastrointestinal environment.\(^{[18]}\) As an example of the PMMA-based materials, we fabricated poly(butyl methacrylate-co-methyl methacrylate), P(BMA-co-MMA) microparticles, with MW of 150 kDa and reported \(T_g\) of 52 °C by the manufacturer (Millipore Sigma, St. Louis, MO), using similar procedure explained above. As shown in Figure 2C and Figure S2E (Supporting Information) isolated particles with uniform size were formed with high resolution. PVAc is another polymer of interest for biomedical applications due to its high biocompatibility with tissues, body fluid, and blood.\(^{[19]}\) Similarly, uniform PVAc (with MW of 100 kDa and reported \(T_g\) of \(\sim 38\) °C)\(^{[20]}\) microparticles were produced as shown in Figure 2D and Figure S2F (Supporting Information). Of note is that the same Fold was repeatedly used for fabrication of all these shape-specific particles of diverse materials without any noticeable change in its performance and resolution of the obtained particles, confirming the excellent retainment of the additive within the PDMS matrix and hence maintaining its extremely nonwetting properties. To further investigate the ability of Fold for creation of identical particles over extended use, we used the same Fold to repeatedly replicate the same cubic structures. Notably, particles obtained after 40 cycles were identical to their initial replicas (Figure S3, Supporting Information) contradictory to the residuals remaining in PDMS mold after a few uses.\(^{[21]}\) Combined, the ability of IMPRESS technique to mold a range of high MW polymers of different chemical compositions with high fidelity provides strong evidence for its robustness and versatility.

Delivery of fragile biologics is a significant medical challenge and requires advanced drug carrier design to maintain the therapeutics’ viability depending on the administration routes.\(^{[22]}\) We have recently developed a technique, stamped assembly of polymer layers (SEAL), that combines \(\mu\)TM technique and thermal bonding to enable fabrication of 3D, multilayered, fillable microparticles.\(^{[23]}\) The core–shell morphology with a built-in reservoir is designed to confine the biologics within the core and protect them before their release inside the body. Particles made of PLGA, a bulk-degrading polymer, release their cargo in a pulsatile fashion at a desired time ranging from few days to months. Thus, the technique offers a promising platform for self-boosting immunization, in which the burst release of the cargo replaces the bolus shots. Additionally, it could also serve as a promising approach for cancer treatment especially for hard-to-reach tumors.\(^{[24]}\) As well as oral delivery of fragile biologics for enhanced bioavailability.\(^{[25]}\) Briefly, SEAL involves the fabrication of particle bases by heat-assisted \(\mu\)TM, as explained above (Figure 1A), in which the formed particles are delaminated onto a flat glass substrate. The second layer, particle caps, are then assembled by a layer-by-layer sintering process under microscopic alignment, in which sealing of the particles is achieved by thermal bonding of the two layers brought into contact and upon brief heating to just above the polymer \(T_g\) (Figure 1A). Although this method is elegant and successful in fabricating complex, multilayered, 3D microdevices, its practicality is still limited. This is largely due to the formation of several scum layers upon the addition of each layer interconnecting the features at multiple conjunctions. For their future applications as drug/vaccine-carrier microdevices, individual features need to be cut precisely. This not only results in a cumbersome cutting step, but also can compromise particle integrity especially at higher MWs where rubbery nature of the polymers makes it difficult to cut through. More importantly, this can prove challenging when injecting the particles, as the scum layer residues, or “skirts” around the cut particles, can potentially clog the needle and lead to dose variability.\(^{[26]}\) This reduces the throughput of the method and limits its clinical translation.

To further demonstrate applicability of IMPRESS technique for fabrication of 3D, multilayered features, we generated discrete, two-layered PLGA core–shell microparticles. While using a PDMS mold led to formation of embossed films with interconnected and undesired features (Figure 2E), a large array of discrete, and sealed PLGA microparticles were formed on the coated glass using IMPRESS technique (Figure 2F). Higher magnification image (Figure S4A, Supporting Information) further demonstrates the high quality of the replication. 3D microstructures at varying sizes and morphologies can be designed by different master molds. To show the breadth of the IMPRESS technique, particles at varying sizes and morphologies were generated (Figure 2G–L). Figure 2G and Figure S4B (Supporting Information) show well-formed, small, 190 \(\mu\)m cubic and sealed individual PLGA microparticles generated using IMPRESS, while Figure S4C,D, Supporting Information show the formation of a skirt around particles made using the PDMS mold. These smaller dimension microparticles were made using PLGA with MW = 19 kDa, in order to show the versatility of the IMPRESS technique for PLGA of various MWs (19–141 kDa). PLGA at this relatively low MW tends to be brittle and the scum layer could break upon peeling off the mold. However, the skirt remaining around the individual particles could still be problematic for their injection. Finally, to show the generality of the technique to create microfeatures of any shape, a large array of two-layered “MIT” letters as well as a curved flag-like PLGA (~141 kDa) microstructures were fabricated (Figure 2H). As can be clearly seen from Figure 2H, no scum layer was formed over a large array of microstructures. Finally, Figure 2I,J shows optical images of isolated PLGA (141 kDa) microparticle bases at various dimensions made with IMPRESS technique. An array of PLGA microparticles filled with a fluorescently labeled blue dye (Alexa Fluor 647-labeled 10 kD dextran) and sealed with corresponding caps is also depicted in Figure 2K. The drug loading capacity depends on particle dimension as well as wall thickness. Particles shown in Figure 2I–L have theoretical loading ranging from 24% for small geometry (160 \(\mu\)m) and about 8% for larger outer dimension
(Figure 2K,L) which was further increased up to about 37% (Figure 2J) by decreasing the wall thickness in half while keeping the overall dimension the same. This tunable loading capacity is an advantageous of core–shell platform compared with traditional emulsion-based particles. Interestingly, the ability of IMPRESS technique to generate features of varying sizes reflects its unique property in controlling the adhesion and friction between the pattern and template. This allows modulation of the surface adhesion properties, simply by changing FRCP segment ratio or concentration of the additive in the blend (discussed in depth in the Supporting Information). As a result, it can overcome the current tribological issues with traditional imprint lithography.[45] The surface composition could be altered so that each layer of the features is transferred onto a substrate or remained on the recessed areas of the Fold (Figure S5, Supporting Information), an important feature for the fabrication of multilayered features. This indicates for patterns with complex internal structure (e.g., particle base versus cap) that have higher contact area with the Fold compared to the substrate and hence higher friction and adhesion forces relative to the Fold, the surface composition can be manipulated for easy release of particles from the Fold. Such tunability, unique to the IMPRESS technique, allows to flexibly engineer microparticle size and shape even with complex internal structures.

Another potential advantage of IMPRESS technique is straightforward harvesting of the formed features. Particles fabricated by the IMPRESS technique can be separated from the coated glass slide easily by a tweezer (Video S1, Supporting Information), without the need for cutting them individually, while particles fabricated using traditional soft lithography were inseparable from the substrate without cutting through the scum layers with a scalpel (Video S2, Supporting Information). Arrays of particles can be removed from the slide simply by sliding a razor blade across the substrate. Figure 2L clearly demonstrates the harvested particles on the tip of the razor blade. Indeed, with IMPRESS method, harvesting a large array of isolated particles with different sizes and shapes was achieved just by gliding a blade across the substrate as shown in Figure S6A–D (Supporting Information). However, in case of PDMS mold, the presence of a thick embossed layer, makes particle harvesting difficult even when excessive mechanical force was applied (Figure S6E,F, Supporting Information). The results offer conclusive evidence for the ability of IMPRESS technique to generate isolated, and harvestable objects as a result of extreme nonwetting properties of the templates. Additionally, the simple mechanical harvesting procedure reflects the potential for continuous fabrication process of microstructures with various shapes and dimensions. Taken together, these discrete and complex 3D microfeatures with excellent control of size and geometry from a wide selection of high MW polymeric materials illustrate the versatility, breadth, and robustness of the IMPRESS technique.

As mentioned above, the core–shell particles can potentially serve as a promising platform for single-injection immunization due to its burst release profile at predetermined time intervals mimicking bolus shots. For practical applications, the polymer material could be altered to adjust the degradation time, matching the timeframe of the desired vaccine.[24] The particles generated using IMPRESS platform are expected to retain the same release profile of the cargo as those made by classical PDMS mold due to the well enmeshment of the FRCP additive within the Fold enabling a residue-free microstructure replication. As such, toxicity profile depends solely on the molded materials and hence is expected to be similar for the IMPRESS-fabricated PLGA particles to that of traditional soft lithography-fabricated microstructures. To confirm this, PLGA microparticles at two different MWs (19 and 75 kDa) were fabricated and filled with a fluorescently labeled dextran, as a model drug for tracking the release, using a picoliter dispensing apparatus (Sciennon, France), and incubated at 37 °C in PBS. Particles made of PLGA at MW of 19 and 75 kDa were released in vitro at 9 ± 0 and 35 ± 1 d, respectively (Figure 3A), with no measurable leakage prior to release. A similar trend was observed when particles were injected subcutaneously into mice flanks. Particles composed of PLGA 19 and 75 kDa released the dye after 9 ± 2 and 40 ± 3 d in vivo, respectively, as signified by an increase in fluorescence upon release, monitored by in vivo imaging system (IVIS) (Figure 3B). Similar release profiles have been reported for particles made using the PDMS molds,[24] suggesting that the IMPRESS technique does not affect the particle release profile and leaching additive out of the template is highly unlikely. Further to that point, no inflammation or signs of toxicity were found at the injected site. Collectively, these results confirm the ease of adaptation of IMPRESS technique to the standard soft lithography technique.

To further examine the potential impact of the IMPRESS technique in drug delivery field, we demonstrate an immediate implementation of this approach beyond creation of isolated features. While microfabrication techniques are gaining considerable attention over the past decade in the drug delivery field, high complexity, cost associated with the materials, facilities, and processing as well as process throughput are still limiting factors hindering the commercialization of such promising technologies.[46] One of the utmost challenges is loading therapeutics into the micron-scale reservoir within the device with high precision and loading efficiency.[47] Although picoliter-volume ink/biojet dispensing machines can be used to fill microparticle arrays with desired volume,[48] throughput of inkjet printing is relatively low since a nozzle is used for dispensing cargo in the particles one by one in a serial fashion. Additionally, to reach the therapeutic window required for clinical dose, multiple deposition cycles are required. This lengthy process of filling and drying can also compromise the viability of sensitive biologics due to cargo instability. Additionally, rapid evaporation of biologically active agent solution (i.e., coffee-ring effect) within the small particle core can have adverse effect on cargo stability. Hence, larger number of the devices are required to achieve a therapeutic dose for a given drug. The dispensing machines are also subject to off-target filling due to droplet instability that can adversely affect the sealing process of the microparticle. An efficient technique that enables parallel loading of large array of microselves in a single step with high accuracy and compatibility with different cargos has not yet been demonstrated. Such method can significantly ease the scalability and extend the use of microfabrication technique for drug delivery.

Here, taking advantage of the extremely nonwetting properties of the template, we developed an innovative stencil-based method to selectively fill internal microreservoir within the
particles in a precise and rapid manner. The technique can be integrated to simplify the manufacturing process of drug-loaded microdevices and obviate the need for additional infrastructure and time-consuming filling process. For this, first, two complementary Folds were fabricated to make the particle base, one with protruding and the counterpart with recessed features (Figure 4A). A PLGA (19 kDa) film was placed between the Folds. The optical clarity of the prepared Fold allows for accurate overlaying of the features. The Folds were then optically aligned and mated with the pillar features and heated up briefly to 75 °C to allow the polymer to fill the cavities within the Fold. After being cooled down to about room temperature, the Folds were clamped and placed in vacuum oven at 120 °C for 2 h. Due to extremely nonwetting properties of the templates, excess polymer was wicked away upon pressing with the clamps, leaving behind an array of discrete particles with an internal reservoir remaining inside the Fold (Figure 4A). The array of particles was then filled by using a fluorinated stencil mask, made by soft lithography (described in the Experimental Section). The fabricated mask was aligned to the microwells and served as a cover for the PLGA sidewalls, shielding them from undesired wetting during the filling step, as it could lead to improper sealing of the particles. A drop of fluorescently labeled dextran was placed on the mask and was pressed against a flat Teflon sheet and into the microwells. Upon a slight downward press, the particle array was filled instantaneously (Figure 4C). Due to extremely hydrophobic nature of the Fold, the aqueous liquid is localized directly into the microdevice reservoirs minimizing drug waste. The particles were then sealed using the polymer cap lids, leaving discrete sealed particles on the glass slide after removing the Fold. This “screen printing” technique presented here allows for instant filling of the cargo directly into device cavity suggesting its potential for fragile biologics. Additionally, this proof-of-concept design reflects the potential of IMPRESS for high-throughput loading of micro-particles and hence the possibility to translate to scalable and

Figure 3. Release from IMPRESSED-fabricated PLGA (19 and 75 kDa) microparticles filled with Alexa Fluor 647-labeled 10 kD dextran. A,B) In vitro pulsatile release of particles incubated at 37 °C (normalized average for n = 10). C,D) In vivo release of particles (normalized average for n = 8). The third row represents images of mice collected with IVIS after injection of single PLGA particle at each mouse flank (error bars show standard error of the mean).
automated processing. It also suggests that engineering surface chemistry and material properties can expand the applicability of microfabrication and could offer new opportunities for overcoming the limitations of traditional drug delivery devices (e.g., isolated array of microneedles to circumvent bed of nail effect[50]).

In conclusion, this work demonstrates that IMPRESS is a simple, versatile, and practical method for fabrication of discrete microfeatures with controlled size and morphology from a wide range of biorelevant polymers. It also enables fabrication of isolated, 3D, multilayered particles. The adhesion properties could be controlled to promote the release of Fold for features with any complex internal structures. The fact that particles can be made directly from polymers of nearly any MW and the ease of adaptation of the technique to standard soft lithography method hold promise for its practicality and clinical translation. We envision that this fabrication method can open up promising routes for creating advanced multi-faceted drug delivery devices and further expanding the microfabrication technique from diagnostics to drug discovery and delivery.

Figure 4. A) Fabrication of PLGA particles and selective filling with a fluorescently labeled dextran using a non-wetting, stencil mask; optical images of PLGA particles B) before and C) after filling.
2. Experimental Section

Materials: See Supporting Information for expanded list of materials.

Polymer Synthesis and Characterization: P(DMS-r-PFDA) was synthesized using free radical polymerization. A total of 20 g PDMS macromonomer and PFDA (at desired ratios: 15 and 25% PFDA, named FRCP15 and FRCP25, respectively) was dissolved in toluene (50 mL). Azobis(isobutyronitrile), AIBN, (20 mg) was added as the initiator. The flask was sealed and purged with nitrogen for 30 min and then heated under stirring at 70 °C for about 14 h. 4-Methoxy phenol, MEHQ, (1 g) was added to terminate the reaction. Copolymers were recovered by precipitation in methanol and purified three times in methanol to remove any residual monomers. The polymer was air-dried under a fume hood overnight and further dried in a vacuum oven at 50 °C.

Synthesis of Folds: A cellenONE nanoliter dispenser (Scienion, France) was used to fill the particles with a 50 mg mL⁻¹ solution of Alexa Fluor 647-labeled 10 kD dextran (Life technologies, Carlsbad, CA), a model drug. Microparticle cores were filled with 2.6 mL solutions over one cycle. In Vivo Release Kinetics: Filled and sealed particles (n = 8) were each placed into 100 μL of phosphate buffer saline (PBS, pH = 7.4) in a lobind microcentrifuge tube (Eppendorf, Hamburg, Germany), vortexed for 15 s and centrifuged for 10 s on a bench top centrifuge. The tubes were then wrapped with an aluminum foil and incubated on a shaker at 37 °C. Release of the fluorescent dye was monitored every 1–3 d depending on particle composition by analyzing the supernatant fluorescence at 650/680 nm.

In Vivo Release Kinetics: Same type of particles examined for in vitro study was also used for in vivo release experiment in female SKH-1 white mice (Charles River Laboratories, Wilmington, MA). All procedures were approved prior to beginning in vivo experiments by Committee on Animal Care (CAC) of Massachusetts Institute of Technology (CAC protocol #: 1019-061-22). Ten days prior to injection, mice were switched to an alfafa-free purified rodent diet (Harlan Laboratories, Madison, WI) to reduce intestinal autofluorescence. Mice were anesthetized for injection by continuous inhalation of 3% isoflurane. Each mouse (n = 5) was injected subcutaneously with one particle at each rear flank using an 18-gauge Monoject filter needle (Covidien, Dublin, Ireland) in approximately 100 μL of 15 mg mL⁻¹ of 4000 cP methylcellulose (Sigma Aldrich). Mice were imaged using a PerkinElmer Spectrum In Vivo Imaging System (IVIS, Hopkinton, MA) two to three times a week depending on the expected release time. At each imaging time point, mice were anesthetized and placed on the heated imaging platform. Fluorescent images were then taken using 640/700 nm excitation/emission filter sets with an F-Stop setting of 2 and subject height of 1.5 cm in Living Image 4.4 software. Cumulative release was normalized to the maximum and minimum total fluorescence in the region of interest corresponding to a particular particle’s complete release and background signal, respectively. Because fluorescence dropped after release due to biological clearance, values after the highest signal was achieved were set to 100% in Figure 3C,D. Release time was considered to be the day on which fluorescence achieved half of its final maximum value above background. For visualization purposes, images were prepared using 3 × 3 smoothing, a binning setting of 4, and reported as radiant efficiency ([pW/cm²/sr]/[μW/cm²]). Prior to release, particle-associated fluorescence was on the order of background autofluorescence, likely due to self-quenching and/or desiccation; however, the signal increased substantially upon release and spreading.

Particle Characterization: High-resolution images of particles were collected using a scanning electron microscope (SEM). Samples were first coated with a thin layer of Au/Pd using a Hummer 6.2 Sputtering System (Anatech, Battle Creek, MI) to avoid charging and then imaged using a JSM-5600LV SEM (JEOL, Tokyo, Japan) with an acceleration voltage of 5 kV.

MICROMOLD FABRICATION: Patterned silicon master molds were created using photolithography, as reported previously. The master molds were then replicated PDMS by soft lithography. To minimize the adhesion of PDMS to the silicon wafer in subsequent steps, the wafer was salinized using trichloro(1H,1H,2H,2H-perfluorooctyl)silane, PFOS. The vapor deposition was achieved by placing the wafer along with a few drops of PFOS in a vacuum desiccator for at least 30 min. Next, PDMS and curing agent were mixed at 7:1 ratio, poured onto silicon master mold, and degassed under vacuum for 1 h. A thin PDMS mold was then produced by attaching three coverslips to the end of a glass slide as spacers and pushing down against the silicon mold while curing at 150 °C for 1 h. Fluorinated molds, Folds, were fabricated following the same procedure with the addition of 0.25–2 wt% FRCP to the elastomer base. The filling mask was made by pouring PDMS blend with added 0.5 wt% of FRCP15 within the gap of silicon wafer master mold clamped against a Teflon slide without any spacers to create the voids.

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Polymer Microtransfer Molding: To generate microscale features by PDMS molds, PDMS molds were silanized by PFOS for at least 30 min to promote delamination of the features from the particles. A polymer film was made by casting a solution of 20% w/w in acetone on a glass slide using a Dr. blade and drying at 45 °C overnight. To make the particle base, the film was placed on a glass slide, pressed against PDMS mold and compressed under a spring-loaded clamp in a 120 °C oven under high vacuum until pattern transfer is complete (≈7 h). After cooling, the features were delaminated from the mold and transferred onto the glass slide. The particle caps were generated by placing a Teflon film between the glass slide and polymer film and clamping against the mold. This will yield features within the patterned cavities of the silanized PDMS mold, which can then be removed upon the sintering process during sealing.

To generate particles using Folds, no silanization step was necessary and particles were delaminated easily from the Fold. Particle fabrication in Folds followed similar steps as in PDMS molds except that the glass slide was coated with a 40% w/v polymer solution (PDMS, 2.5–5% w/v of copolymer additive) and curing agent at 2000 rpm for 2 min and then cured in a similar condition to the Fold. Features were made similarly by placing a PLGA film on a coated glass slide and were clamped against the Fold.

Fabrication of Multilayered Structure by Aligning and Sintering: The multilayer features were aligned with high precision using a house-made system comprised of a microscope with a rotation integrated positioning stage retrofitted with a Peltier heater, temperature controller, relay, and voltage source. The stage was connected to a vacuum line to hold glass slide containing the features. The second layer of the microfeatures was held onto a metal frame connected to the vacuum line and was then screwed on top of the rotating stage. After optical alignment of the layers under microscope, the layers were brought into contact and heated at 45–52 °C, depending on the polymer T_g, for up to 10 min until they fused.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

R.L. and A.J. are the holders of the patent describing fabrication of core-shell microparticles titled “Micromolded or 3-D printed pulsatile release vaccine formulations” (U.S. patent application 10/300136). A.J. and R.L. are the holders of another related patent titled “Microdevices with complex geometries,” (filed 13 September 2017; application number US 62/558172).

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords

drug delivery, multilayered microparticles, random copolymers, soft lithography, surface-segregating polymers

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