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Potent Engineered PLGA Nanoparticles by Virtue of Exceptionally High Chemotherapeutic Loadings

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Abstract

Herein we report the fabrication of engineered poly(lactic acid-co-glycolic acid) nanoparticles via the PRINT® (Particle Replication In Non-wetting Templates) process with high and efficient loadings of docetaxel, up to 40% (w/w) with encapsulation efficiencies >90%. The PRINT process enables independent control of particle properties leading to a higher degree of tailorability than traditional methods. Particles with 40% loading display better *in vitro* efficacy than particles with lower loadings and the clinical formulation of docetaxel, Taxotere®.

Keywords

PLGA; nanoparticle; docetaxel; PRINT®

Advances in nanotechnology have brought about innovative solutions to disease therapy and new methods for the fabrication of drug delivery vehicles. Liposomes, conjugates, and other traditional nanocarriers have established the field of advanced drug delivery and provided valuable information on the ability of nanocarriers to increase the efficacy of therapeutics by reducing systemic exposure, increasing therapeutic concentrations at the disease site and modifying exposure times by providing sustained release of therapeutics.[1-3] The efficacy shown by these traditional nanocarriers has led to the next generation of drug delivery vehicles, polymeric particles, which have a greater ability to protect cargo, program release and design multifunctionality.[4] Of particular interest is poly(lactic acid-co-glycolic acid) (PLGA), a biocompatible, bioabsorbable polymer which has already shown promise in

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Supporting Information **Available.** Materials and methods. DSC thermogram of pure docetaxel. Docetaxel release profiles. This information is available free of charge via the Internet at http://pubs.acs.org/.

medical applications and can easily be tailored to vary release and degradation. Various methods for fabricating PLGA particles, including emulsions, precipitations, spray drying, and flow focusing, are currently under investigation by a large number of research groups.[5-10] A substantial volume of literature has been dedicated to investigating the effects of process parameters on particle properties. They have demonstrated that solvent(s), emulsifier(s), and particle composition affect particle size and cargo release, which are themselves interrelated.[11-15] Varying one factor systemically is consequently a challenge. Furthermore cargo encapsulation is an issue with traditional methods. These processes generally involve a two phase system: a solvent and an anti-solvent for PLGA. When incorporating cargo, affinity between phases must be considered and inevitably some cargo is lost to the anti-solvent phase, most typically water. This has lead to the study of encapsulation efficiency, a parameter describing the ability of a process to encapsulate cargo in the particle. This is important because poor encapsulation results in lower drug loadings which in turn limit the particles' therapeutically effective dose.

The PRINT® (Particle Replication In Non-wetting Templates) process, a soft lithography platform, simplifies fabrication and particle design with the unique ability to control size and shape independent of process variables. In addition to size and shape control, the PRINT process creates truly monodisperse particles, is not limited to spheres, is scalable and allows for easy encapsulation of a wide range of cargos including hydrophilic or hydrophobic therapeutics, biologicals, proteins, siRNA, and imaging agents.[16-18] Soft lithography in general offers a great alternative for the fabrication of highly engineered particles, but has not been used for the fabrication of cargo containing PLGA nanoparticles. Some investigation into non-spherical PLGA microparticles has been conducted including the fabrication of micron scale PLGA features by imprint lithography which can contain a reservoir[19,20] and the deformation of PLGA microspheres into a wide variety of non-spherical shapes using a film stretching technique[21]. Recently a hydrogel template method was demonstrated for the fabrication of PLGA particles. Microparticles were fabricated in a wide range of sizes and shapes and were loaded with high levels of Felodipine, a hypertension therapeutic. The feature resolution and loading abilities on the nanoscale has yet to be investigated, though the fabrication of drug free 200 nm cubes was demonstrated.[22] These technologies clearly point to the desire to have more control over PLGA drug carriers, but have yet to produce nanoparticles with independent control over all key parameters and proven efficacy as drug delivery vehicles.

To demonstrate the efficacy of PLGA PRINT nanoparticles as drug delivery agents, we have applied them to the delivery of a chemotherapeutic agent, docetaxel. Docetaxel is a cytotoxic antimicrotubule agent which has been approved for the treatment of cancer in the form of Taxotere®, a formulation of docetaxel in water and ethanol with poloxomer 188 (Tween 20) for stabilization. Docetaxel has shown promise over doxorubicin, paclitaxel and fluorouracil, however, it has dose limiting toxicities associated with systemic delivery, making it a prime candidate for improvement through encapsulation in a delivery vehicle.[23] Several groups are investigating the use of PLGA particles as delivery vehicles for docetaxel (Table 1). Using a block copolymer of PLGA and PEG to form micelles which encapsulate docetaxel in their hydrophobic core is the most common approach with maximum loadings at 15%. These methods have generated particles which have increased the therapeutic efficacy of docetaxel *in vitro* and *in vivo*. By further extending control over particle properties and by increasing the drug loading potential, we hope to further improve the *in vivo* efficacy of docetaxel.

Versatile Particle Fabrication

The PRINT process is a soft lithography platform based on a perfluorinated polyether elastomer. The low surface energy of this material prevents wetting so while the cavities are filled the land area remains clear resulting in isolated particles (Scheme 1). Filling the mold requires a phase transition or solvent evaporation step and in the case of PLGA a melt-solidification transition is employed. A film of polymer is briefly heated in contact with a mold during which the polymer flows into the cavities, pulled by capillary forces, and then solidifies as the polymer returns to room temperature. The particles can then be transferred from the mold to any flat surface or to an excipent layer by briefly reheating in contact with the desired surface. Once the particles are transferred from the mold they can be collected mechanically or, in the case of an excipent layer, the excipent is simply dissolved releasing the particles from the surface.

PLGA PRINT particles have been fabricated in a wide variety of sizes and shapes (Figure 1). The shape of each particle is based solely on the shape of the cavity in the elastomeric mold. Particles can be fabricated with high aspect ratios as shown in A and C at two different sizes. High aspect ratio particles carry a higher payload when compared to spherical particles of the same critical dimension. If, for example, an 80 nm sphere and an 80 × 320 nm cylinder can both extravasate through a 100 nm pore then the high aspect ratio particle, which is 6 times larger in volume, has the opportunity to deliver more drug. Particles can be fabricated with surface texture as in E where particles have ridges on the sides. In this way particles with varying surface areas and surface roughness can be created. These surface features could be used to alter drug release or to affect movement under flow conditions, both in air flow (inhalation) and in the bloodstream. Similarly fenestrations can be included in the pattern as demonstrated in F where hex nut shaped particles were fabricated. Particles which approximate the classical spherical shape of most other methods can also be generated as shown in D. These particles are generated from a dome shaped master and do have a flat spot. They can be further rounded by an additional heating step to remove this flat spot. Since size and shape are completely independent of process parameters the same particle geometry can be created with a variety of polymer molecular weights, polymer lactic acid to glycolic acid ratios, solvent systems, stabilizers, and cargos. This leads to an ability to tailor the particle beyond what is currently available. It is known that size and shape can be used to achieve specific cell uptake and biodistribution profiles.[32-34] Furthermore it is established that molecular weight and copolymer ratio can be used to achieve varied release and degradation profiles. Using the PRINT process these four parameters can be individually tailored. And unlike traditional techniques that require a stabilizer, the choice of which affects particle formation, stabilizers used in the PRINT process are added after particle fabrication and can be chosen independently. Herein poly(vinyl alcohol) (PVOH) was chosen as a stabilizer.

High Drug Loading

In addition to exquisite control over the physical properties of the particle, the PRINT process allows for complete control over particle composition. The polymer molecular weight and lactic acid to glycolic acid ratio can be varied and incorporating cargo is straightforward. The “second phase” in this process is a perfluoropolyether network which is both oleophobic and hydrophobic so partitioning into this material is low.[35] This has led to the ability to achieve good encapsulation with loadings much higher than possible with traditional methods. In the literature (Table 1) maximum docetaxel loading is 15% with encapsulation widely varying dependent not only on the particular fabrication method, but on the specific parameters used. Here we have loaded 200nm × 200nm cylindrical particles with 0 – 40% docetaxel (w/w) using the same processing parameters for all compositions.

Encapsulation efficiency is >90% for all compositions. Others have cited lowered encapsulation efficiency as drug loading increases[26], however, with the PRINT process loading does not affect encapsulation nor does it affect the particle's physical properties. The largest dimension of the 200nm × 200nm cylinder is approximately 280nm and the smallest 200nm which is reflected in the size measured by dynamic light scattering (DLS) and is consistent across all compositions (Table 2). The charge is negative, typical of PLGA nanoparticles, and also consistent. SEM shows the particles are a homogeneous size and shape within and between compositions (Figure 2). The ability to create the same outward physical properties with different compositions in a homogeneous population of particles is going to generate investigations into interesting dosage questions that traditional methods can not explore. Of particular interest is the difference between administering the same overall drug dose in a high mass of particles with low drug loading versus a low mass of particles with high drug loading. The ability to make a particle with the exact same outward physical characteristics removes the additional variables of size and size distribution which would cloud this study by affecting biodistribution. The availability of more dosing options may provide a better chance of increasing efficacy in patients.

In addition to outward physical properties, the morphology of the particles was also examined. Differential scanning calorimetry (DSC) was used to examine the T_g of the polymer and the state of the docetaxel. As Figure 3 shows the T_g of the polymer is consistent across all compositions, varying by only 5°C between particles with 0% and 40% docetaxel. All samples also show a similar endothermic peak accompanying the T_g commonly assumed to be a physical aging peak in polymers Furthermore the docetaxel, which when crystalline has a melting point of 172°C (Figure S1), is not in a crystalline state even at very high docetaxel loadings as demonstrated by the lack of a melting peak. Since the particles are generated from a solid state solution, the docetaxel is evenly distributed within the polymer and does not crystallize. As the sample is heated and the polymer is melted, rearrangement can occur and the docetaxel appears to be crystallizing during the run as indicated by the exothermic peak at 168°C present in all samples containing docetaxel.

In Vitro Drug Delivery Efficacy

In addition to being able to load a cargo efficiently, it is essential that the cargo is able to be released while maintaining activity and achieving efficacy. A comparison of toxicity among particles containing 0 – 40% docetaxel on SKOV3, ovarian carcinoma, cells is shown in Figure 4. Particles without drug are non-toxic as expected from the biocompatible, bioabsorbable nature of the polymer. PLGA PRINT nanoparticles containing docetaxel exhibit dose dependent toxicity and are toxic in sub-nanomolar docetaxel concentrations. These results demonstrate not only that the PRINT process allow for high encapsulation of docetaxel, but that the docetaxel is released from the particle, can be delivered to its desired cellular location, and is unharmed by the process conditions. The particles are compared to the clinically administered form of docetaxel, Taxotere®. Slightly less toxicity compared to Taxotere is seen with particles containing 10% and 20% docetaxel while particles containing 30% and 40% docetaxel show higher toxicity at the same docetaxel concentration (Table 3). Particles with 40% docetaxel have an IC_{50} almost an order of magnitude lower than Taxotere and 30 times lower than particles loaded with 10% docetaxel, the comparable loading to traditional methods. Since release rates are equal for all four PRINT docetaxel compositions (Figure S2), it suggests higher docetaxel loadings could be important to increase efficacy at a lower total dose and further investigation into the effects of higher loadings on toxicity are warranted.

Conclusions

Herein a novel method for the fabrication of highly engineered PLGA particles has been demonstrated. This method, built on the PRINT® technology platform, allows for the fabrication of particles of almost any shape and size independent of process parameters. These sizes and shapes can be used to affect cell uptake, biodistribution, and flow characteristics. Cargo is easily encapsulated without the necessity of adjustments to the process. High and efficient drug loadings have been demonstrated with docetaxel, a potent chemotherapeutic. These particles have shown high toxicity *in vitro*; investigation of efficacy *in vivo* is underway. The PRINT process allows for the fabrication of highly engineered PLGA particles with the ability to encapsulate high therapeutic loadings efficiently, showing great promise as a tailorable drug delivery system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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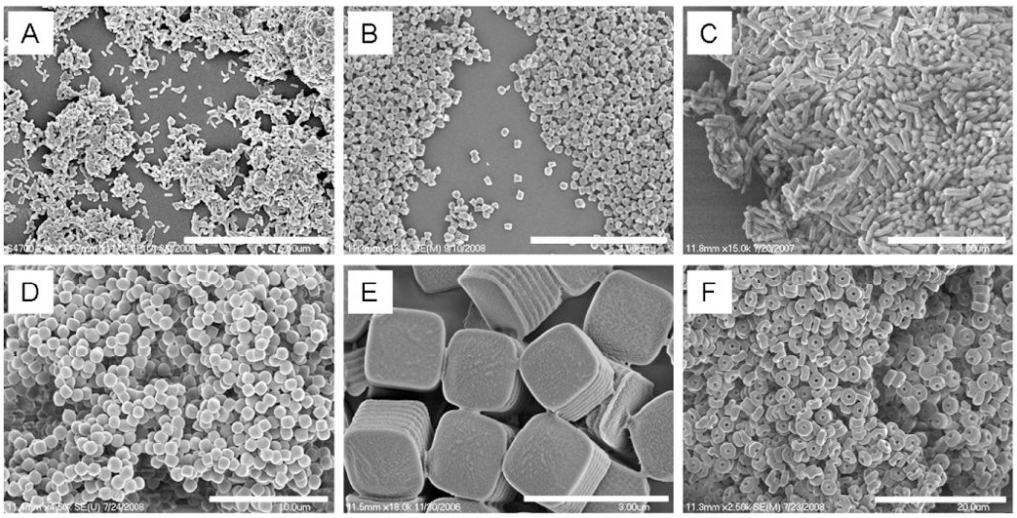


Figure 1.
PLGA nano- and microparticles fabricated by the PRINT process. (A) 80nm × 360nm cylinders, (B) 200nm × 200nm cylinders, (C) 200nm × 600nm cylinders, (D) 1 μm sphere approximates, (E) 2 μm cubes with ridges, and (F) 3 μm particles with center fenestrations. Scale bars: (A) 5 μm, (B) 4 μm, (C) 3 μm, (D) 10 μm, (E) 3 μm, and (F) 20 μm.

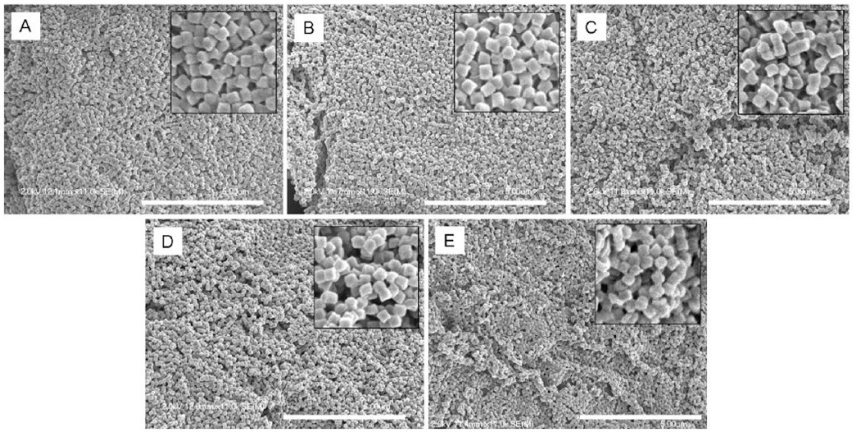


Figure 2.
SEM images of cylindrical 200nm × 200nm PLGA PRINT nanoparticles containing varying amounts of docetaxel: A) 0%, B) 10%, C) 20%, D) 30%, E) 40%. All scale bars are 5 μm. Inset images are a magnification of a portion of the image to the same scale for more detail.

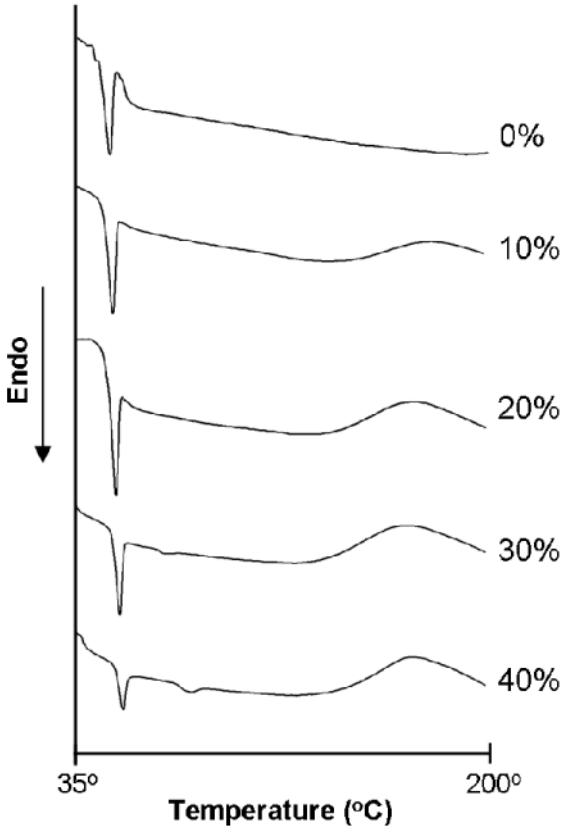


Figure 3.
DSC thermograms of PLGA PRINT nanoparticles with different docetaxel loadings (both x- and y-axis are scaled the same for all thermograms).

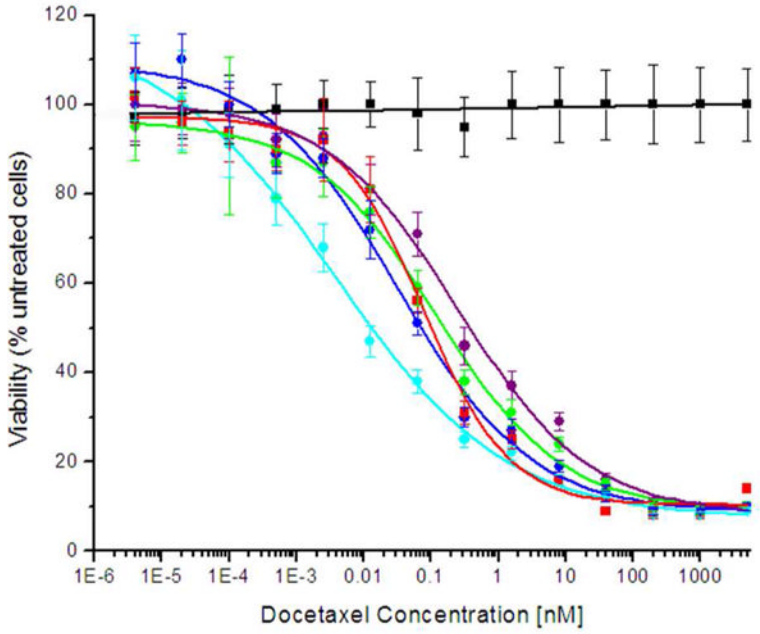
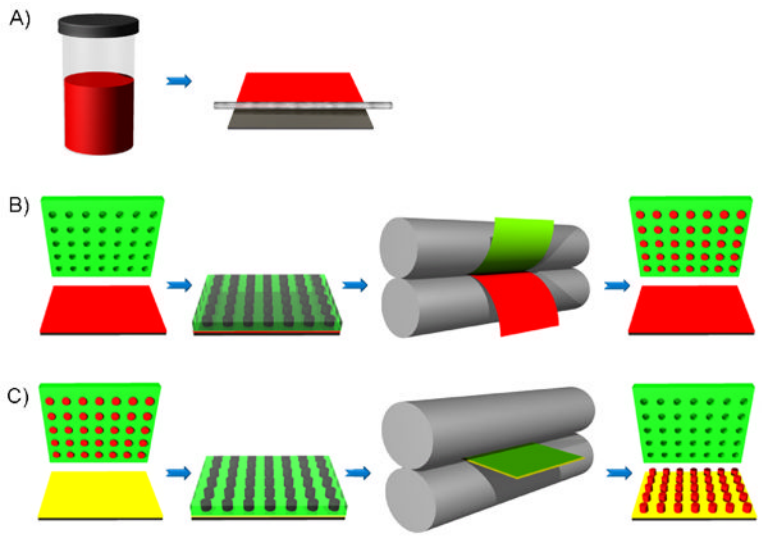


Figure 4.

SKOV3 cell viability after 72 hour exposure to Taxotere® and PLGA PRINT nanoparticles containing various docetaxel weight percents: 0% docetaxel (black), 10% docetaxel (purple), 20% docetaxel (green), 30% docetaxel (dark blue), 40% docetaxel (light blue), and Taxotere® (red). Blank particles (0%) were dosed at equal particle concentrations to 10% docetaxel containing particles (i.e. the highest particle dose).



Scheme 1.

The PRINT process: (A) Delivery Sheet Casting: PLGA and docetaxel are dissolved in DMF and DMSO (4:1 solvent ratio) to create a true solution (red). A mayer rod is then used to draw a film from this solution on a PET substrate. The solvent is removed under heat generating a solid state solution film referred to as the delivery sheet, as it will deliver the composition to the mold. (B) Particle fabrication: a perfluoropolyether elastomeric mold (green) in brought into contact with a PLGA (red) film, passed through a heated nip (gray) and split. The cavities of the mold are filled. (C) Particle harvesting: a filled mold is brought into contact with a high energy film or excipent layer (yellow) and passed through the heated nip without splitting. After cooling the mold is removed to reveal an array of particles on the high energy film or excipent layer.

Table 1

Encapsulation of docetaxel in PLGA/PLA particles.

Fabrication Method	Matrix	Theoretical Loading	Encapsulation Efficiency	Ref
Emulsion	PLGA	0.5-1%	17-23%	24
	PLA	0.5-1%	11-22%	24
	PLGA-mPEG	2%	77-83%	25
	PLGA and PLGA-mPEG	2%	38-85%	26
	PVP-b-PLGA	4%	>95%	27
	PLGA-mPEG	6%	26%	26
	PLGA-lecithin-PEG	10%	62%	28
	PLGA	11%	70%	15
	PLGA/Poloxamer188	11%	88%	15
Nanoprecipitation	PLGA-PEG	10-15%	21-51%	29
Film Rehydration	PEG-b-PLA	12%	98%	30
Ultrasonication	NGR-PLA-PEG	5-15%	95-98%	31

Table 2

Encapsulation efficiency of PLGA PRINT nanoparticles at varying drug loadings (w/w) measured by HPLC and physical characterization by DLS.

Theoretical Loading	Encapsulation Efficiency (%)	Absolute Loading (%)	Size (nm)	Zeta Potential (mV)
0%	--	0 ± 0	263 ± 5	-22.6 ± 0.6
10%	93 ± 11	9.3 ± 1.1	256 ± 10	-19.8 ± 0.8
20%	95 ± 5	19 ± 1	246 ± 2	-22.3 ± 0.3
30%	99 ± 7	29.7 ± 2.1	247 ± 4	-19.6 ± 0.3
40%	99 ± 3	39.6 ± 1.2	251 ± 1	-21.8 ± 0.2

Table 3

IC_{50} values for docetaxel loaded PLGA PRINT Nanoparticles and Taxotere.

	IC_{50} [nM of Docetaxel]
Taxotere	0.103
10% Docetaxel PLGA PRINT	0.379
20% Docetaxel PLGA PRINT	0.158
30% Docetaxel PLGA PRINT	0.072
40% Docetaxel PLGA PRINT	0.013