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Zapped Assembly of Polymeric (ZAP) Nanoparticles for Anti-Cancer Drug Delivery

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Abstract

The starting hypothesis for this work was that microwave synthesis could enable the rapid assembly of polymers into size-specific nanoparticles (NPs). The Zapped Assembly of Polymeric (ZAP) NPs was initially realized using poly(lactic-co-glycolic acid)-poly(ethylene glycol) (PLGA-PEG) block copolymers and distinct microwave reaction parameters. A library of polymeric NPs was generated with sizes ranging from sub-20 nm to 350 nm and low polydispersity. Select ZAP NPs were synthesized in 30 seconds at different scales and concentrations, up to 200 mg and 100 mg/mL, without substantial size variation. ZAP NPs with diameters of 25 nm, 50 nm, and 100 nm were loaded with the chemotherapeutic paclitaxel (PXL), demonstrated unique release profiles, and exhibited dose-dependent cytotoxicity similar to Taxol. Incorporation of d-alpha tocopheryl polyethylene glycol succinate (TPGS) and PLGA_{33k} allowed for the production of a sub-40 nm NP with an exceptionally high loading of PXL (12.6 wt%, ca. 7 times the original NP) and a slower release profile. This ZAP NP platform demonstrated scalable, flexible, and tunable synthesis with potential toward clinical scale production of size-specific drug carriers.

Graphical Abstract.

Illustration of the Zapped Assembly of Polymeric (ZAP) nanoparticles processing by the microwave heating of PLGA-PEG, PLGA, TPGS, and PXL in solvent followed by cooling to produce nanoparticles with exceptionally high loading of PXL (12.6 wt%, ~7 times higher than the original PLGA-PEG NPs).

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Conflicts of interest

There are no conflicts to declare.

†Electronic Supplementary Information (ESI) available.

Introduction

To minimize toxicity and side effects attributed to chemotherapy, the utilization of nanoparticles (NPs) has demonstrated enhanced delivery of drugs to cancerous tissue.^{1–3} The phenomenon of enhanced passive targeting is dictated by the size and composition of the nano-therapeutic. For effective passive targeting, nanoparticles should be larger than 5.5 nm to avoid renal clearance;⁴ yet, small enough to avoid clearance by the reticuloendothelial system (<200 nm).⁵ NPs in this size range can accumulate in solid tumors through the enhanced permeability and retention (EPR) effect in which solid tumors display leaky vasculature and dysfunctional lymphatic drainage. Large NPs (100 nm) have been noted to have minimal permeation in the tumor while small NPs (20 nm) have been shown to have minimal retention within the tumor.⁶ This behavior can depend on the type and stage of the tumor. To effectively target a particular cancer, a repertoire of particle sizes to select from would be advantageous.

A large majority of nanoparticles administered into blood are cleared by the mononuclear phagocyte system (MPS). The MPS rapidly clears charged NPs that have adsorbed serum proteins markers.⁷ To avoid protein adsorption, NPs are often decorated with neutral, stealth-like polymers such as poly(ethylene glycol) (PEG). Addition of PEG minimizes serum protein markers on the surface of NPs, which in turn extends circulation half-life of NPs.⁸ NPs with extended circulation times have the potential for enhanced drug delivery to tumors.

To use nanoparticles for cancer therapies, there are additional challenges in achieving effective delivery of drugs. For example, nanoparticles must be able to incorporate poorly-soluble chemotherapeutic drugs. The stable incorporation of chemotherapeutic drugs has been accomplished with a variety of NPs, e.g. liposomes, viral vectors, carbon nanotubes, PRINT® particles, micelles, and polymeric NPs.^{9–17} Poly(lactic-co-glycolic acid) (PLGA)-based nanoparticles have been extensively utilized for drug delivery in cancer therapy.^{18–22} PLGA is an FDA-approved biocompatible and biodegradable copolymer that allows for modular drug release rates from hours to months²³ by varying the molecular weight and ratio of glycolic acid to lactic acid. Notable methods to produce PLGA nanoparticles include nanoprecipitation, nanoemulsification, microfluidics, and Particle Replication In Non-wetting Templates (PRINT®).^{24–31}

Another method to produce nanoparticles involves microwave heating.^{32,33} This approach has provided smaller nanoparticles with lower polydispersity indices (PDIs) due to rapid,

homogeneous, and efficient dielectric heating.^{34,35} Our approach exploits the advantages of microwave synthesis for the Zapped Assembly of Polymeric (ZAP) NPs. Tuning reaction parameters in microwave synthesis enabled the creation of a library of ZAP PLGA-PEG NPs with distinct and ultra-small sizes. From this library, specific nanoparticles were selected for scale-up, characterization, and translation into cancer therapies. The chemotherapeutic paclitaxel was incorporated into ZAP nanoparticles and exhibited unique release rates. Drug-free ZAP NPs were biocompatible with human cervical carcinoma (HeLa) cells while paclitaxel-loaded NPs elicited dose-dependent cytotoxicity comparable to Taxol.

Materials and methods

Materials

PLGA-PEG block copolymers were purchased from Akina, Inc. Paclitaxel was obtained from LC Laboratories (165 New Boston Street, Woburn, MA). DMSO, empty PD-10 columns, calcium chloride, sodium chloride, Slide-A-Lyzer G2 Dialysis Cassettes, Parafilm M™ Laboratory Wrapping Film, and HPLC-purified water were purchased from Thermo Fisher Scientific, Inc. Polyoxyl 20-stearyl ether (Brij 78) was acquired from Uniqema (Wilmington, DE), d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS) was purchased from Eastman Chemicals (Kingsport, TN), DSPE-mPEG_{2k} was acquired from Creative PEGWorks, PLGA (50:50, 33 kDa) was purchased from Lakeshore Biomaterials, and Miglyol 812 was obtained from Sasol (Witten, Germany). HPLC grade acetonitrile was obtained from Acros. All other solvents, Zonyl® FS-300, Pluronic® F-127, and Sepharose CL-4B were purchased from Sigma. Nanoparticle fabrication occurred in a Biotage® Initiator Classic microwave synthesizer. Dynamic light scattering took place in a Zetasizer Nano ZS Particle Analyzer (Malvern Instruments, Inc.). Paclitaxel analysis was performed using High-Performance Liquid Chromatography (Agilent 1260 – Infinity). Particle quantification was performed by thermogravimetric analysis (Discovery TGA, TA Instruments). Transmission Electron Microscopy was carried out using JEOL JEM 1230 TEM.

Synthesis of nanoparticles

PLGA-PEG block copolymers were first dissolved in acetone. An aliquot of this solution (containing 0.5, 5, 25, 50, or 200 mg) was added to a 0.5–2.0 mL or 10–20 mL microwave reaction vial. The solvent was evaporated, yielding a polymeric film. For the specific reaction parameters utilized, a particular solvent (0.5 mL or 20 mL) was added to the vial, which was charged with a magnetic stir bar and sealed with a microwave cap. The vial was then loaded into the microwave synthesizer for a given temperature and time using the very high absorption setting and fixed hold time (countdown started after reaching the target temperature). Reaction parameters are listed in Table S1–S7. For single additive NPs, 5 mg of 2:1 (by weight) PLGA_{5k}-PEG_{2k}: Brij 78, Tween 80, TPGS, PLGA_{33k}, DSPE-mPEG_{2k}, Pluronic F-127, or Zonyl FS-300 was used. For miglyol 812, 5, 2.5, 1.25, and 0.625 wt% was incorporated in 5 mg of PLGA_{5k}-PEG_{2k}; for calcium chloride, 100 mM was used with 5 mg PLGA_{5k}-PEG_{2k}. For formulations with two additives, the compositions are listed in Table S8. All nanoparticles were synthesized in triplicate.

Characterization of nanoparticles

The size and zeta potential of ZAP nanoparticles was evaluated using Zetasizer Nano ZS Particle Analyzer (Malvern Instruments, Inc.). For samples in water, aliquots were placed directly in a disposable microcuvette for analysis. For samples in organic solvent mixtures, 0.5 mL of the particle dispersion was added to 0.6 mL of water, which was then aliquoted into a glass cuvette with round aperture. Three size measurements were taken for each particle sample using automatic measurement duration and 173° backscatter general purpose analysis model at 25 °C in the particular solvent. The zeta potential was measured using a clear disposable zeta cell with the sample in 10 mM NaCl, automatic duration time, three measurements, and the Smoluchowski approximation. Particle concentration was measured by thermogravimetric analysis (Discovery TGA, TA Instruments). The TGA method was: ramp at 30 °C/min to 150 °C; hold isothermal for 10 minutes; ramp at 20 °C/min to 25 °C; hold isothermal for 2 minutes. TEM samples were prepared by dispensing nanoparticles in water on 400 mesh copper formvar/C-coated grids and staining with 2% uranyl acetate.

Characterization of nanoparticle stability over time

For the stability of particles over time, size measurements were taken after 1, 2, 3, and 4 weeks from the initial measurement using three different particle batches. 50 nm and 100 nm nanoparticles were stored at 0.2 mg/mL and 25 nm nanoparticles were stored at 1 mg/mL particle concentration at 23 °C in 1.5 mL Eppendorf tubes in deionized water and dispensed into a disposable microcuvette for size analysis at each time point. Also, nanoparticles were incubated at 5 mg/mL in cell culture medium or plasma at 37 °C and an aliquot was taken at each time point for Zetasizer analysis.

Synthesis and characterization of paclitaxel nanoparticles

Paclitaxel was dissolved in acetone, from which an aliquot containing 0.55 mg was charged into a 0.5–2.0 mL microwave reaction vial. To this vial, an aliquot of 5 mg of polymer solution in acetone was added. The solutions were mixed and then evaporated to yield a drug-containing polymeric film. Particles were then synthesized as described above and purified from free drug using size exclusion chromatography (SEC). SEC columns were prepared by adding Sepharose CL-4B to empty PD-10 columns and water was used as the eluent. All nanoparticles were synthesized in triplicate and were analyzed by Zetasizer, TGA, and HPLC. For HPLC analysis, aliquots were diluted 3-fold with acetonitrile. The method for HPLC analysis utilized 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in acetonitrile (solvent B). A gradient from 95:5 to 0:100 of A:B was used over 20 minutes, followed by running pure B for 2 minutes. Then, a gradient from 0:100 to 95:5 of A:B over two minutes was implemented followed by running for 3 minutes at 95:5 A:B. The wavelengths studied for paclitaxel, which eluted between 12.92–13.15 minutes, were 250 nm and 230 nm.

Drug release studies

Nanoparticles were dispensed in 0.5 mL Slide-A-Lyzer G2 Dialysis Cassettes. These were placed in a 2 L beaker containing PBS at 37 °C. Aliquots were taken at 15 min, 30 min, 1 h,

2 h, 4 h, 8 h, 24 h, 48 h, and 72 h. These aliquots were diluted 3-fold with acetonitrile and analyzed by HPLC.

***In vitro* cytotoxicity**

HeLa cells were purchased directly from the American Type Culture Collection prior to initiation of these studies. All cell-based assays were performed utilizing cells at passage numbers ranging from 6–16. HeLa cells were seeded in 200 μ L of media [D-MEM (high glucose), 10% fetal bovine serum (FBS), 0.1 mM MEM Non-Essential Amino Acids (NEAA), 2 mM L-glutamine, 1% Pen-Strep, (optional)] at a density of 5000 cells per well into a 96-well microtiter plate. Cells were allowed to adhere for 24 hours (hr) and then incubated with PLGA-paclitaxel ZAP NPs and paclitaxel at paclitaxel concentrations ranging from 10 μ M to 0.0061 μ M for 72 continuous hr or dosed for 4 h on cells, washed, and then incubated for 68 h at 37 °C in a humidified 5% CO₂ atmosphere. After the incubation period, all medium/ZAP NPs were aspirated off cells. One hundred microliters of fresh medium was added back to cells followed by the addition of 100 μ L CellTiter-Glo® Luminescent Cell Viability Assay reagent. Plates were placed on a microplate shaker for 2 minutes (min), then incubated at room temperature for 10 min to stabilize luminescent signal. The luminescent signal was recorded on a SpectraMax M5 plate reader. The viability of the cells exposed to PLGA-paclitaxel ZAP NPs (and paclitaxel) was expressed as a percentage of the viability of untreated cells.

Results and discussion

PLGA-PEG block copolymers were selected as the particle matrix due to their physical properties and FDA approved biocompatible and biodegradable components. This copolymer provides a hydrophilic corona with PEG to promote prolonged circulation and evasion of the reticuloendothelial system. Furthermore, PLGA offers a hydrophobic, glassy core for particle stability and incorporation of hydrophobic drugs. A range of molecular weights were chosen to offer various physical properties. The molecular weights for PLGA-PEG were: 55k-5k, 20k-5k, 10k-5k, and 5k-2k. To synthesize ZAP NPs, a film of PLGA-PEG was prepared on a microwave vial. A particular solvent was added to the film and then the vial was subjected to specific reaction parameters to form the nanoparticles by microwave heating.

The reaction parameters tested in microwave synthesis were time, temperature, solvent composition, and polymer molecular weight. The times evaluated ranged from 1 second to 2 hours while temperature was varied from 60 °C to 180 °C. A variety of solvent mixtures were tested using pure water or different weight percentages of acetonitrile, acetone, isopropanol, ethanol, N,N-dimethylformamide, dimethyl sulfoxide, and tetrahydrofuran. The mechanism for ZAP particle formation was envisioned to proceed via dispersion and nanoparticle formation through heating followed by vitrification through cooling.

Here, a library of ZAP PLGA-PEG NPs was synthesized by varying microwave reaction parameters (Figure 1, Table 1). The largest particles (>300 nm) were obtained with PLGA_{55k}-PEG_{5k}. Furthermore, the longest reaction times were required for the PLGA_{55k}-PEG_{5k} copolymer. This may be attributed to more time needed for dispersion of the high

molecular weight, glassy polymer into the solvent from a solid phase. On the other end of the spectrum, the smallest NPs (<30 nm) were obtained in the shortest reaction times and lowest temperatures for the low molecular weight copolymer. PLGA_{5k}-PEG_{2k} may readily disperse in the solvent at lower temperatures, thus avoiding the need to be exposed to elevated temperature for prolonged periods of time. Accessing sub-20 nm NPs is comparable to state-of-the art microfluidic synthesis of small PLGA-PEG NPs.²⁸

For simplicity, PEG blocks will be left out of the remaining polymer identifiers unless necessary. It was desired to obtain 100 nm, 50 nm, and 25 nm NPs to use for scalability, stability, and drug loading and delivery studies. To obtain these sizes, different PLGA-PEG copolymers and microwave reaction parameters were systematically screened (Figure S1). It was found that 100 nm NPs were obtained with PLGA_{20k} using 50% ACN and 150 °C in 1 min; 50 nm NPs with PLGA_{10k} using 35% ACN and 120 °C in 30 sec; and 25 nm with PLGA_{5k} using 35% ACN and 90 °C in 30 sec. These three identified particles were then used for the subsequent experiments.

The scalability of ZAP PLGA NPs was demonstrated by synthesis of PLGA_{10k} 50 nm NPs at 0.5, 5, 25, 50, and 200 mg scales in 30 seconds (Figure 2a). The nanoparticle diameters were statistically similar using a t-test. In addition to scalability, flexibility of the platform was demonstrated by synthesis of PLGA NPs at different concentrations, specifically, 10 mg/mL, 50 mg/mL, and 100 mg/mL (Figure 2b). Nanoparticle diameters differed less than 10% between concentrations.

The room temperature storage stability of 25 nm, 50 nm, and 100 nm NPs was studied over time by measuring their size in one week intervals over one month (Figure S2). PLGA_{10k} 50 nm and PLGA_{20k} 100 nm particles changed less than 2 nm over 4 weeks while PLGA_{5k} 25 nm NPs increased 3 nm. The changes in size at each week for each particle compared to the initial time point were not statistically significant as determined by a t-test. The stability of PLGA_{20k} NPs under physiological conditions was studied by monitoring size and ζ -potential of nanoparticles incubated in plasma and cell culture medium (CCM) at 37 °C (Figure 2c,d). CCM consisted of 10% FBS supplemented DMEM. The diameter of PLGA_{20k} NPs differed less than 10% of the initial diameter when incubated in CCM and plasma out to 48 h. The zeta potential of nanoparticles in plasma was initially much more negative than in CCM, which may be due to the higher concentration of biological components and proteins. The zeta potential of nanoparticles in plasma differed less than 5% of the initial value over 72 h.

To translate these particles into cancer therapies, paclitaxel was encapsulated in NPs. Paclitaxel was charged into nanoparticle formulations at 10 wt% and subjected to different reaction conditions. PXL was encapsulated in PLGA_{5k} 25 nm NPs at 1.9%, PLGA_{10k} 50 nm NPs at 3.5 wt%, and PLGA_{20k} 100 nm NPs at 6.7%. The incorporation of paclitaxel did not notably affect zeta potential or size of the nanoparticles (Table 2) as they were statistically similar as determined by a t-test. The zeta-potential of the NPs ranged from -3 mV to -6 mV. Release of paclitaxel from 25 nm, 50 nm, and 100 nm NPs at 37 °C in phosphate buffered saline (PBS) was studied out to 72 h (Figure 3a). The release kinetics of NPs followed an exponential decay with $R^2 > 0.99$. The rate of drug release increased with

decreasing particle size and molecular weight. Furthermore, the time at which 50% drug was release increased with particle size, which was ~3 h for 100 nm NPs, ~2 h for 50 nm NPs, and ~1 h for 25 nm NPs. Similarly, the weight percent (loading) of drug increased with particle size. The drug release profiles were characteristic of burst release followed by diffusion and biodegradation: higher molecular weight PLGA-PEG and larger NPs degrade slower and have smaller surface area to volume ratios, resulting in slower release of drug.

Human cervical carcinoma (HeLa) cells were dosed with paclitaxel, paclitaxel-encapsulated ZAP PLGA nanoparticles, and blank ZAP PLGA NPs (Table 2). Specifically, cells were dosed with PLGA_{5k} 25 nm, PLGA_{10k} 50 nm, and PLGA_{20k} 100 nm NPs. Particles were dosed on cells followed by 72 h incubation (Figure 3b) and demonstrated dose-dependent cell death. The half maximal inhibitory concentration (IC₅₀) was calculated for paclitaxel-loaded NPs (Table 3). The IC₅₀ increased with decreasing PXL NP size, polymer molecular weight, drug loading, and t_{50%}. The IC₅₀s of NPs were similar to that of PXL where the IC₅₀ of PLGA_{20k} 100 nm NPs was slightly lower than that of PXL. In addition to dosing and incubating for 72 h, PXL NPs were dosed for 4 h on cells, washed, and then incubated for 68 h (Figure S4). This dosing resulted in greater IC₅₀ values (Table 3) as there may be less exposure to the NP and drug. Lastly, cytocompatibility of blank NPs was evaluated as a function of equivalent paclitaxel dosing concentrations. Cell viability was maintained greater than 85% across all dosing concentrations and particle samples (Table 3, Figure S5).

Although ZAP PLGA-PEG NPs showed effective drug delivery, the loading of PXL was relatively low and the release was rather quick for the 25 nm NPs. Therefore, in an effort to increase loading and tune the release of the 25 nm nanoparticles, different additives were explored. Additives included amphiphilic surfactants, pure polymer, salt, lipids, and a triblock copolymer (Figure S6). The additives investigated were (1) single hydrophobic chain amphiphilic surfactants Tween 80, TPGS, and Brij 78, (2) pure PLGA_{33k} copolymer, (3) triglyceride oil miglyol 812 and DSPE-mPEG_{2k}, (4) perfluorinated hydrophilic surfactant Zonyl® FS-300, (5) calcium chloride salt, and (6) triblock copolymer Pluronic® F-127. Aggregated and polydisperse samples were found for Pluronic F-127, CaCl₂, Zonyl FS-300, DSPE-mPEG_{2k}, and miglyol 812 (Figure S7) while well-defined, monomodal nanoparticles were encountered with Tween 80, TPGS, Brij 78, and PLGA_{33k}. These observations may be attributed to compatibility of the additive with the PLGA-PEG block copolymer.

For compatibility, a single hydrophobic and hydrophilic domain was necessary for surfactants. In contrast, Pluronic F-127 has two hydrophilic poly(ethylene glycol) domains and one hydrophobic poly(propylene glycol) domain while DSPE-mPEG_{2k} has two hydrophobic tails. Perfluorinated hydrophilic surfactant Zonyl FS-300 was most likely chemically incompatible with hydrophobic domains. Addition of calcium chloride could have led to inter-particle crosslinking and aggregation. Miglyol 812 may not have been able to fully incorporate and stabilize in the PLGA domains due to its oily nature. Use of pure PLGA_{33k} copolymer as an additive to PLGA-PEG yielded well-defined NPs with a larger size, which may be due to expansion of the hydrophobic core.

An optimal composition was established by combining PLGA_{5k}-PEG_{2k}, PLGA_{33k}, and single hydrophobic chain amphiphilic surfactants (composition details in Table S8). All additive ZAP PLGA-based NPs showed monomodal size and ζ -potential distributions (Figure 4a,b). The synthesis of these additive NPs at different concentrations showed a size dependence on concentration (Figure 4c). Specifically, NP size mostly became smaller at higher concentrations. This may be rationalized by LaMer's nucleation theory in which high concentrations result in more nucleation events and lead to smaller sizes instead of growing into larger NPs by a ripening process.^{36,37} The addition of surfactant and pure PLGA copolymer appeared to have facilitated increased nucleation events. Also, scalability of ZAP PLGA-based NPs with Tween 80 was demonstrated out to 100 mg (Figure 4d). The NP size was found to be larger at smaller scales, which may similarly follow LaMer's nucleation theory.

These additive NPs were then loaded with PXL to determine the effect on encapsulation and release. When 10 wt% PXL was charged into formulations, all additive PLGA-based ZAP NPs showed higher loadings of PXL than the original 25 nm NPs. This may be attributed to the aliphatic groups and solubilizing PXL (Figure 5a). Still, the additive NPs maintained sub-40 nm diameters (Table 4). The hydrophile-lipophile balance for Brij 78 and Tween 80 are similar as were their corresponding PXL loadings (around 5 wt%). When 15 wt% PXL was charged into the formulation, Brij 78 and Tween 80 showed polydisperse size distribution while TPGS exhibited well-defined, monomodal size distribution (Figure S8, Table S10). TPGS reached the highest loading (12.6 wt% of PXL), which was approximately 7 times higher than the original 25 nm NP. The aromatic groups on the hydrophobic vitamin E portion of TPGS may be able to solubilize PXL and pi stack. To our knowledge, this is the highest PXL loading for a sub-40 nm PLGA-based NPs in the literature. The closest comparison is PCL-PEG micelle NPs that show ~15% loading and a diameter of 44 nm.³⁸

The release of PXL from additive NPs was studied in PBS at 37 °C over time (Figure 5b). TPGS showed the fastest release of PXL for all the additives. TPGS is a solid at room temperature and has a melting temperature of 38 °C, right around physiological conditions; this melting may result in expulsion of drug while Tween 80 is already liquid and Brij 78 has a melting point of ~45 °C. Still, for each additive nanoparticle, the time at which half of the drug was released was longer compared to the original 25 nm PLGA-PEG block copolymer nanoparticle. Specifically, the $t_{50\%}$ was ~ 7 h, 6 h, and 3 h for Tween 80, Brij 78, and TPGS additive NPs, respectively.

Conclusions

Microwave heating allowed for tailored synthesis of PLGA-PEG NPs with specific sizes through the Zapped Assembly of Polymeric (ZAP) NPs. A library of PLGA-PEG NPs was synthesized by varying the microwave reaction parameters. State-of-the art specialized microfluidics has achieved the synthesis of nanoparticles with diameters down to 13 nm. Here, we approach that minimum size with the demonstrated synthesis of 18 nm NPs in 30 seconds. The scalability and versatility of this platform was exemplified by the synthesis of NPs from 0.5 mg to 200 mg scales. In addition, this platform provided flexibility by

enabling the synthesis of similar-sized NPs at different and high concentrations, up to 100 mg/mL. Nanoparticles produced from this platform showed stability over time under storage and physiological conditions. From the library, 25 nm, 50 nm, and 100 nm NPs were loaded with the chemotherapeutic paclitaxel and exhibited distinct drug release profiles. Blank NPs were cytocompatible with HeLa cells while drug-loaded NPs elicited dose-dependent cell death comparable to Taxol. To increase loading and alter release profiles for small NPs, different additive were incorporated into the formulation. Addition of TPGS and PLGA_{33k} enabled the synthesis of sub-40 nm NPs with an exceptionally high loading of PXL (12.6 wt %) and a slower release profile. Future efforts may involve pursuing a continuous, scaled-up system and evaluating the effect of NP size on *in vivo* efficacy for treating tumors. The microwave-assisted synthesis approach disclosed herein provides the fundamentals for rapidly developing size-specific PLGA-based NPs that effectively encapsulate and deliver paclitaxel. We envision that further iterations on this approach may enable additional particle compositions, NP sizes, surface chemistries, and encapsulation of a variety of cargos for cancer therapies and other applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Fig. 1. Nanoparticle diameters of ZAP PLGA-PEG NPs synthesized by microwave irradiation as a function of reaction parameter numbers listed in Table S6, TEM image of ZAP PLGA_{20k}-PEG_{5k} NPs (scale bar = 100 nm), and number percentage distribution of diameters for select NPs of different molecular weights.

Fig. 2.

(a) Scalability of ZAP PLGA_{10k} NPs by synthesis at 0.5 mg to 200 mg scales. (b) Flexibility of ZAP NPs by synthesis at different concentrations. (c) Stability of ZAP PLGA_{20k} NPs by size under physiological conditions in cell culture medium (CCM) and plasma over time at 37 °C. (d) Stability of ZAP PLGA_{20k} NPs by ζ-potential in CCM and plasma at 37 °C.

Fig. 3.

(a) Release profile of paclitaxel (PXL) from PLGA_{5k} 25 nm, PLGA_{10k} 50 nm, and PLGA_{20k} 100 nm NPs incubated in PBS at 37 °C. (b) Percent viability of HeLa cells dosed with paclitaxel and paclitaxel-loaded PLGA_{5k} 25 nm, PLGA_{10k} 50 nm, and PLGA_{20k} 100 nm nanoparticles at different doses after 72 h incubation.

Fig. 4.

(a) Size distribution by volume fraction for ZAP PLGA-based NPs with different additives. (b) Zeta potential distribution for additive NPs. (c) Diameter of additive NPs synthesized at different NP concentrations. (d) Diameter of ZAP PLGA-based NPs with Tween 80 synthesized at different scales.

Fig. 5.
(a) Weight percent of PXL encapsulated in additive NPs versus original NP formulation (25nm). (b) Release of PXL from NPs incubated in PBS at 37 °C over time.

Table 1

Diameter and polydispersity of select nanoparticles fabricated from different polymers using specific reaction times corresponding to Figure 1 inset number distributions.

Polymer	Reaction time	Nanoparticle diameter (nm)	Polydispersity Index
PLGA _{5k} -PEG _{2k}	30 seconds	18.2 ± 1.8	0.14 ± 0.04
PLGA _{10k} -PEG _{5k}	30 seconds	32.3 ± 2.3	0.14 ± 0.04
PLGA _{20k} -PEG _{5k}	1 minute	72.4 ± 7.4	0.10 ± 0.03

Table 2

Physicochemical characterization of blank and paclitaxel (PXL) drug-loaded NPs.

Drug-Polymer	Diameter (nm)	ζ_p^a (mV)	PXL loading (wt%)	$t_{50\%}^b$ (h)
Blank-PLGA _{5k}	26.6 ± 3.7	-3.4 ± 1.5	NA	NA
PXL-PLGA _{5k}	22.6 ± 3.0	-3.2 ± 0.9	1.9 ± 0.3	1.2
Blank-PLGA _{10k}	53.4 ± 1.2	-4.0 ± 0.2	NA	NA
PXL-PLGA _{10k}	53.5 ± 2.2	-4.7 ± 1.2	3.5 ± 1.4	2.1
Blank-PLGA _{20k}	100.1 ± 3.0	-5.6 ± 0.5	NA	NA
PXL-PLGA _{20k}	104.3 ± 6.8	-4.8 ± 0.2	6.7 ± 1.6	3.2

^a ζ_p represents zeta-potential

^b $t_{50\%}$ is the time at which 50% of drug is released.

Table 3

Half maximal inhibitory concentration after 72 h or 4 h dosing and 68 h incubation for paclitaxel-loaded particles on HeLa cells and viability of cells incubated with blank NPs.

Sample	72 h IC ₅₀ (nM)	4 h IC ₅₀ (nM)	Cell viability
PLGA _{5k} 25 nm	97.0	910.7	>96% ^a
PLGA _{10k} 50 nm	81.9	872.5	>96% ^a
PLGA _{20k} 100 nm	54.4	570.1	>85% ^a
PXL	58.0	436.4	NA
Cells only	NA	NA	100%

^aRepresented as the minimum percent viable cells from all equivalent drug dosing concentrations for blank NPs.

Table 4

Physicochemical characterization of additive PLGA-based nanoparticles.

Additive	Diameter (nm)	ζ_p^a (mV)	PXL charged; loaded (wt%)	$t_{50\%}^b$ (h)
TPGS	38.8 ± 2.0	-6.4 ± 2.5	15; 12.6 ± 1.1	2.8
Tween 80	26.4 ± 3.0	-3.9 ± 1.1	10; 4.8 ± 1.0	6.9
Brij 78	36.0 ± 2.6	-4.1 ± 0.5	10; 4.8 ± 0.1	6.0

^a ζ_p represents zeta-potential

^b $t_{50\%}$ is the time at which 50% of drug is released.