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Bioactive Hybrid Particles from Poly(D,L-lactide-*co*-glycolide) Nanoparticle Stabilized Lipid Droplets

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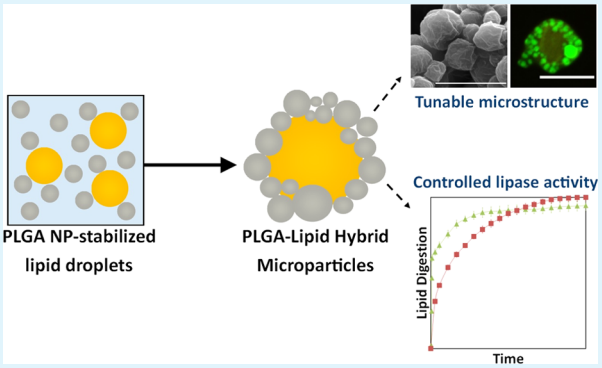
Supporting Information

ABSTRACT: Biodegradable and bioactive hybrid particles composed of poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles and medium-chain triglycerides were prepared by spray drying lipid-in-water emulsions stabilized by PLGA nanoparticles, to form PLGA–lipid hybrid (PLH) microparticles approximately 5 μm in mean diameter. The nanoparticle stabilizer was varied and mannitol was also incorporated during the preparation to investigate the effect of stabilizer charge and cryoprotectant content on the particle microstructure. An in vitro lipolysis model was used to demonstrate the particles' bioactivity by manipulating the digestion kinetics of encapsulated lipid by pancreatic lipase in simulated gastrointestinal fluid. Lipid digestion kinetics were enhanced in PLH and PLGA–lipid–mannitol hybrid (PLMH) microparticles for both stabilizers, compared to a coarse emulsion, in biorelevant media. An optimal digestion rate was observed for the negatively charged PLMH system, evidenced by a 2-fold increase in the pseudo-first-order rate constant compared to a coarse emulsion. Improved microparticle redispersion, probed by dual dye confocal fluorescence microscopy, increased the available surface area of lipid for lipase adsorption, enhancing digestion kinetics. Thereby, lipase action was controlled in hybrid microparticles by altering the surface charge and carbohydrate content. Our results demonstrate that bioactive microparticles composed of versatile and biodegradable polymeric particles and oil droplets have great potential for use in smart food and nutrient delivery, as well as safer and more efficacious oral delivery of drugs and drug combinations.



KEYWORDS: *poly(lactic-co-glycolic), PLGA, lipid formulation, lipid digestion, lipase, hybrid microparticles*

INTRODUCTION

A major challenge facing biomaterials research is the development of smarter and more powerful carriers that effectively deliver bioactive molecules, while retaining biocompatibility. Formulating complex pharmaceuticals, such as proteins, peptides, and lipophilic molecules, with nanocarriers is often required to overcome physicochemical limitations and, thereby, attain their full therapeutic potential.[1] Lipid based formulations, such as liposomes, lipid nanoemulsions, and solid lipid nanoparticles, along with polymeric nanoparticles are two of the most investigated nanocarrier systems for pharmaceuticals.[2] Lipid systems have been widely used in drug delivery applications due to high biocompatibility, favorable pharmacokinetic profiles,[3] and ease of fabrication.[4] However, issues arise due to instability, insufficient drug loading, and burst release of encapsulated drug molecules.[5,6] Alternatively, polymeric nanoparticles can be fabricated to encapsulate molecules with poor solubility and permeability, control drug release, and sustain circulation while providing higher stability in biological fluids.[7] However, the biocompatibility of many polymeric nanoparticles is not as attractive as lipid systems.[8,9] Consequently, it is desirable to engineer nanostructured lipid carriers that combine the advantages of lipid systems with polymeric nanoparticles, while minimizing the physicochemical and biological limitations of the two nanocarriers.

A wide range of polymer–lipid nanocomposites have been reported recently with the aim to address multifaceted drug delivery challenges.[10,11] The most extensively fabricated type of hybrid particle is lipid coated poly(D,L-lactide-*co*-glycolide) (PLGA) particles[12] and these are commonly assembled via a two-step synthesis method whereby anionic PLGA nanoparticles are mixed with cationic liposomes at the desired ratio.[1,13] In this case, electrostatic attraction between the two components is the major driving force for the formation of polymer–lipid hybrids.[14] Single-step fabrication techniques also exist which minimize batch-to-batch variation of physicochemical properties, and hence drug delivery performance.[15] This approach utilizes phospholipids as emulsifiers in the nanoparticle synthesis, resulting in the self-assembly of lipid coated polymer nanoparticles.[11]

Poly(D,L-lactide-*co*-glycolide) (PLGA) is an FDA approved biodegradable polymer that has received the most extensive attention in the field of polymer–lipid hybrids due to its

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biocompatibility.[16] Lipid coated PLGA nanoparticles and microparticles have demonstrated several potential advantages over conventional delivery systems such as controllable particle size for high uptake, surface functionality for targeted delivery, high drug loading, entrapment of multiple therapeutic agents for combinational therapy, and tunable drug release profiles.[12] However, such systems still face challenges in controlling the rate of drug release from the lipid component, since burst release profiles are often observed from drugs encapsulated within the lipid shell.

Silica−lipid hybrid (SLH) microparticles, a novel nanostructured lipid carrier developed by our group, consist of lipid encapsulated within a three-dimensional porous silica matrix.[17−19] SLH microparticles are prepared by spray drying a silica stabilized emulsion, with the water removal process inducing the controlled aggregation of silica particles into a spongelike matrix.[20] The lipid emulsifier charge impacts the nanostructure of the dry SLH microparticles due to the enhanced stabilizing effect of nanoparticles when a charge neutralization mechanism is operative in the Pickering emulsion.[17] The oral absorption of a number of lipophilic drugs in SLH microparticles has been shown to increase as a result of improved drug solubility, which is attributed to the enhanced and controlled digestion of lipid encapsulated in the three-dimensional silica matrix by the digestive enzyme, pancreatic lipase.[21−23] The increased interfacial surface area of lipid, binding support of hydrophilic silica, and reduced interference effect of digestion products have shown to enhance lipase action in SLH microparticles.[24] However, the exact effect of surface chemistry of the solid matrix support on the digestibility of the encapsulated lipid is not well understood. In addition to this, Yu et al.[25] recently expressed concerns surrounding the in vivo biocompatibility and toxicity of silica nanoparticles with varying particle sizes, geometries, and porosities. Results demonstrated that acute toxicity was mainly influenced by particle porosities and surface characteristics, whereby adverse reactions above the maximum tolerated dose caused organ failure. Consequently, it is desirable to design a novel nanoparticle−lipid hybrid system based on SLH microparticles but without associated toxicity concerns.

The ability to form hybrid microstructures with porous three-dimensional matrixes whereby lipid is encapsulated within a solid matrix is controlled initially by the ability for solid nanoparticles to form a Pickering emulsion with medium-chain triglycerides (MCTs). PLGA nanoparticles with a slight negative surface charge, due to the use of poly(vinyl alcohol) (PVA) as a stabilizer, have shown the ability to impart kinetic stability to a range of nonpolar oils by forming weak interactions with the oil−water interface, creating a physical barrier to drop coarsening.[26] In this paper, we combine the stabilizing effect and controlled delivery characteristics of PLGA nanoparticles with the solubilizing effect of lipid droplets to form dry PLGA−lipid hybrid (PLH) microparticles with novel architecture through the process of spray drying. The effect of preparation parameters, including the influence of PLGA nanoparticle stabilizer charge and the use of mannitol as a cryoprotectant during the water removal step, on the structural properties was characterized using confocal fluorescence and scanning electron microscopy (SEM). The rate of in vivo drug solubilization for poorly water-soluble drugs is controlled by pancreatic lipase mediated digestion kinetics, as the digestion products form mixed micelles which further solubilize the encapsulated drug molecules prior to absorption into the blood.[3] Thus, the bioactivity of PLH microparticles was investigated by determining the influence of particle microstructure on lipase-mediated digestion using an in vitro model. The mechanism of lipase action was controlled in PLH microparticles, and enhanced digestion kinetics were demonstrated for microparticles that readily redisperse and disintegrate in digestion media.

MATERIALS AND METHODS

Materials. Poly(D,L-lactide-*co*-glycolide) (PLGA; lactide:glycolide 50:50, MW = 30 000−60 000 Da), didodecyldimethylammonium bromide (DMAB), and poly(vinyl alcohol) (PVA; MW = 30 000−70 000 Da) were purchased from Sigma-Aldrich (Australia). Medium-chain triglyceride (MCT; Miglyol 812) was obtained from Hamilton Laboratories (Adelaide, Australia), and soybean lecithin (containing >94% phosphatidycholine and <2% triglycerides) was obtained from BDH Merck (Sydney, Australia). Fluorescent dyes, DiIC18(3) (DiI) and coumarin 6, along with ethyl acetate (AR grade), were obtained from Sigma-Aldrich (Australia). Materials used for the lipolysis study, including sodium taurodeoxycholate (NaTDC) 99%, Trizma maleate, type X-E L-α-lecithin (approximately 60% pure phosphatidylcholine, from dried egg yolk), porcine pancreatin extract (activity equivalent to 8 × USP specification), calcium chloride dehydrate, and sodium hydroxide pellets, were also purchased from Sigma-Aldrich (Australia). All chemicals were of analytic grade and used as received. High purity (Milli-Q) water was used throughout the study.

Preparation of PLGA Nanoparticles. Nanoparticles were prepared by a modified emulsion−diffusion−evaporation method developed by Hariharan et al.[27] A 500 mg sample of PLGA was dissolved in 25 mL of ethyl acetate at room temperature for 2 h. Ethyl acetate was selected due to its favorable physical properties over other organic solvents, in particularly its low surface tension with water and low viscosity that imparts high stability on the synthesized nanoparticles by forming particles with smaller diameters.[28,29] The organic phase was added to 50 mL of an aqueous phase containing DMAB (PLGA(+) nanoparticles) or PVA (PLGA(−) nanoparticles) as a stabilizer (250 mg in 50 mL, 0.5%, w/v). The resulting primary emulsion was stirred at 1000 rpm for 3 h and subsequently homogenized at 1000 bar for 5 min using a high-pressure homogenizer (Avestin EmulsiFlex-C5 Homogenizer). Water was added with constant stirring to this nanoemulsion to facilitate diffusion and, finally, evaporation of ethyl acetate, leading to the nanoprecipitation of nanoparticles.

Preparation of Nanoparticle−Lipid Hybrid Microparticles. *Synthesis of PLGA−Lipid Hybrid (PLH) Microparticles.* PLH microparticles were prepared using a two-step process (i.e., homogenization followed by spray-drying of PLGA nanoparticle stabilized emulsions). The initial oil-in-water emulsions were prepared by dissolving 0.6% (w/w) lecithin in 10% (w/w) oil (Miglyol 812), and Milli-Q water was added as the continuous phase. PLGA nanoparticles were dispersed in Milli-Q water, which contained 60% (weight relative to oil content) nanoparticles. The coarse emulsion was tumbled for 12 h after the addition of PLGA nanoparticles prior to being homogenized (Avestin EmulsiFlex-C5 Homogenizer) under a pressure of 1000 bar for five cycles. The PLGA nanoparticle stabilized emulsion was then spray dried (Mini Spray Dryer B-290, BÜCHI Labortechnik AG) to form PLH microparticles under the following conditions: emulsion flow rate, 0.5 mL/min; air flow rate, 0.6 m^3/min; inlet temperature, 60 °C; outlet temperature, 35 °C; aspirator setting, 10.

Synthesis of PLGA−Lipid−Mannitol Hybrid (PLMH) Microparticles. PMLH microparticles were prepared by adding a mannitol dispersion, containing 10% (weight relative to PLGA nanoparticles) mannitol, to the PLGA nanoparticle dispersion. This mixture was then added to the emulsion before homogenization and spray dried following the method described above.

Physicochemical Characterization of PLH Microparticles. *Confocal Laser Scanning Microscopy (CLSM).* Confocal fluorescence imaging analysis was performed by staining the lipid and PLGA

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nanoparticles with DiI and coumarin 6, respectively. Confocal images of the PLGA-stabilized wet emulsion and the spray dried hybrid microparticles were detected at the emission wavelength of 561 nm for DiI (excitation wavelength 540 nm) and 488 nm for coumarin 6 (excitation wavelength 497 nm), which appeared as red and green, respectively (Nikon A1-R microscope).

Scanning Electron Microscopy (SEM). The particle size and surface morphology of PLH microparticles were examined by high resolution analytical scanning electron microscopy (SEM; Quanta 450). Each sample was mounted on double-sided adhesive tape and sputter coated with a platinum layer prior to imaging.

Lipid Loading Content. The lipid loading content of PLH microparticles was determined by thermogravimetric analysis (TGA). The particles were heated at a scanning rate of 10 °C/min from 20 to 550 °C under nitrogen purging. The lipid completely decomposed by 500 °C. Since PLGA also decomposed within this range, the weight loss corresponding to the PLGA component was measured by initially heating spray dried PLGA nanoparticles (in the absence of lipid) within this temperature range. The amount of lipid loaded within the PLH microparticles could be deconvoluted by evaluating the difference in primary derivative weight change peaks between the spray dried PLGA nanoparticles and PLH microparticles. An example of the TGA evaluation is provided (Supporting Information, Figure S1). Mannitol concentration could not be determined using this technique for PLMH microparticles as it decomposed within the same temperature range as PLGA.

Redispersibility Study. The redispersibility properties of PLH and PLMH microparticles were assessed based on changes in droplet size over a period of time as characterized by laser diffraction (DLS) using a Malvern Mastersizer and dynamic light scattering using a Malvern Zetasizer Nano, respectively. Each sample (5 mg/mL powder) was gently stirred in lipid digestion medium following the method of Jang et al.,[30] to determine the change in average particle size over time.

In Vitro Lipolysis Studies. *Preparation of Lipid Digestion Medium.* Fasted state mixed micelles (1.25 mM PC/5 mM NaTDC) were used as lipid digestion medium and were prepared according to the method adapted from Sek et al.[31] Egg lecithin was dissolved in chloroform (5 mL) followed by evaporation of chloroform under vacuum (Rotavapor RE, BÜCHI, Switzerland) to form a thin film of lecithin around the bottom of a 50 mL round-bottom flask. NaTDC and digestion buffer [50 mM Trizma maleate (pH 7.5), 150 mM NaCl, and 5 mM $CaCl_2 \cdot 2H_2O$] was added, and the mixture was stirred for 12 h to produce a transparent (light yellow) micellar solution. Pancreatin extracts (containing pancreatic lipase, colipase, and other nonspecific lipolytic enzymes and proteins such as phospholipase A_2) were prepared by stirring 1 g of porcine pancreatin powder in 5 mL of digestion buffer for 15 min, followed by centrifugation (at 5000 rpm, 4 °C) for 20 min. The supernatant phase was collected and stored at 4 °C until use.

Lipid Digestion Kinetics Studies. The progress of lipid digestion was monitored for 180 min using a pH-stat titration unit (TIM854 Titration Manager, Radiometer, Copenhagen, Denmark) according to the lipolysis protocol as described by Sek et al.[31] A known quantity of sample (equivalent to approximately 200 mg of lipid) was dispersed in 18 mL of buffered micellar solution by stirring continuously for 10 min in a thermostated glass reaction vessel (37 °C). The pH of digestion medium was readjusted with 0.1 M NaOH or HCl to 7.50 ± 0.01. Lipolysis was initiated by addition of 2 mL of pancreatin extract (containing approximately 2000 TBU of pancreatic lipase activity) into the digestion medium. Free fatty acids (FFA) produced in the reaction vessel were immediately titrated with 0.6 M NaOH via an autoburet to maintain a constant pH in the digestion medium at the preset value of 7.50 ± 0.01 throughout the experiment.[21]

The hydrolysis reaction can be treated as a pseudo-first-order process as the rate of reaction is dependent on the concentration of the substrate, due to the excess amount of enzyme added to the digestion media. The pseudo-first-order model can be used to develop a simple rate expression but ignores the effect of interfacial composition on lipase activity.

$$\%H = H_{max}(1 - e^{-kt}) \quad (1)$$

where $\%H$ is percent lipid hydrolysis, H_{max} is maximum achievable lipid hydrolysis, k is the first-order reaction rate constant, and t is time.

RESULTS AND DISCUSSION

Fabrication of Hybrid Particles from PLGA Nanoparticle Stabilized Lipid Droplets. *Synthesis and Characterization of PLGA Nanoparticles.* PLGA nanoparticles were prepared via an emulsion−diffusion−evaporation method using ethyl acetate as the organic solvent. Zeta (ζ) potential and particle size are important parameters that have direct relevance to nanoparticle stability. For greatest stability, ζ potential, either positive or negative, should be high and particle size should be low to facilitate electrostatic repulsion and reduce particle aggregation.[32] DMAB, a double-tailed cationic surfactant, and PVA, a nonionic surfactant, were used to form PLGA(+) and PLGA(−) nanoparticles, respectively. In doing so, the role of stabilizer charge and structure on nanoparticle stability was investigated. DMAB produced nanoparticles with a highly cationic charge (90.5 ± 6.1 mV), whereas PVA produced nanoparticles with an anionic charge (−21.6 ± 3.6 mV). Due to its structure, DMAB has a significantly lower critical micelle concentration (cmc) than PVA, and thereby aggregates at lower concentrations and solubilizes the organic polymer solution more readily than PVA.[33] This led to lower interfacial tension at the organic droplet interface, and therefore PLGA(+) nanoparticles were smaller than PLGA(−) nanoparticles, given by particle sizes of 164 ± 8.1 nm [polydispersity index (PDI) = 0.11] and 257 ± 10.9 nm (PDI = 0.16), respectively (Supporting Information, Table S1). Thus, nanoparticles with the greatest stability were formed when DMAB was used as a stabilizer when compared with PVA.

PLGA Nanoparticle Stabilized Lipid Droplets. Medium-chain-length triglyceride (MCT) droplets stabilized by the phospholipid emulsifier, lecithin, were homogenized to form 210 ± 22 nm emulsion droplets in water. PLGA(+) and PLGA(−) nanoparticles were separately used to stabilize the lipid-in-water droplets. Hydrophobic and electrostatic forces are the fundamental colloidal interactions that influence drop stability for particulate emulsifiers. Particles can only attach to drop surfaces providing that they approach closely enough for the thin liquid film to rupture.[34] Lecithin induced negative charge on emulsion droplets, enabling oppositely charged PLGA(+) nanoparticles to overcome this energy barrier through electrostatic attractive interactions and coat the lipid droplet interface, as previously demonstrated by Whitby et al.[26] This was evident by an increase in particle size from 0.5 to 3.0 μm over a 60 min period (Figure 1), indicating a high degree of droplet−particle coagulation. By contrast, electrostatically repulsive interactions between negatively charged PLGA(−) nanoparticles and anionic emulsion droplets generated an energy barrier to close approach.[35] PLGA(−) nanoparticles demonstrated a weak adsorption on the emulsion droplets, due to hydrophobic interactions, as evidenced by a small increase in particle size from 0.4 to 0.9 μm and minimal aggregation shown in confocal fluorescence images (Figure 1, inset).

Synthesis of PLGA−Lipid Hybrid (PLH) Microparticles. Submicrometer lipid emulsions stabilized by PLGA(+) and PLGA(−) nanoparticles (step A to step B, Figure 2) formed the precursor for the fabrication of PLH microparticles. The consequent nanoparticle-stabilized emulsions were spray dried to form hybrid microparticles approximately 5 μm in mean

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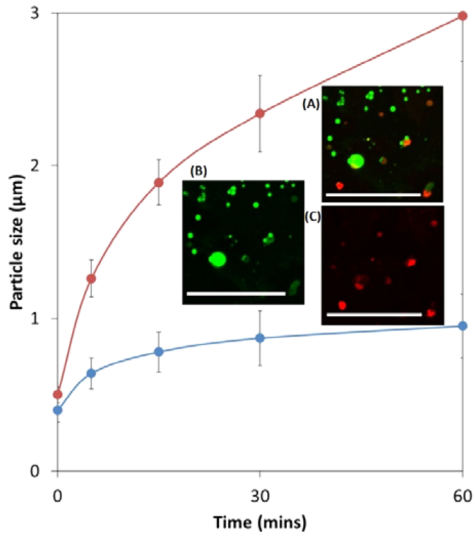

Figure 1. Average particle size as a function of time of MCT emulsion droplets in the presence of (red ●) PLGA(+) nanoparticles and (blue ●) PLGA(−) nanoparticles. (inset) Confocal fluorescence images of negatively charged emulsion droplets in the presence of PLGA(−) nanoparticles. (A) Overlay; (B) λ = 488 nm (PLGA component); (C) λ = 561 nm (lipid component). Scale bars represent 5 μm.

diameter (step E, Figure 2; Table 1). Due to the high concentration of PLGA nanoparticles (60:40 PLGA:lipid wt %), the ζ potential of the stabilized emulsions and the dry PLH microparticles was dependent on the charge of the nanoparticle. Extrapolation of the data gives an isoelectric point between pH 9 and 10 for PLH-1 microparticles and between pH 3 and 4 for PLH-2 microparticles (Supporting Information, Figure S2).

Table 1. Physicochemical Properties of Hybrid Microparticles Composed of PLGA Nanoparticles and Lipid Droplets in the Absence and Presence of Mannitol

sample	av particle size (μm)	ζ potential[a] (μm)	lipid loading level (wt %)
PLH(+)	4.85 ± 0.9	16.2 ± 3.2	37
PLH(−)	4.23 ± 0.7	−29.4 ± 6.4	34
PLMH(+)	5.63 ± 1.2	49.9 ± 7.3	26
PLMH(−)	5.15 ± 1.6	−1.2 ± 1.2	22

[a]Determined in the pH range 7.0−7.5.

The dry powder formed from spray drying PLGA nanoparticle stabilized emulsions was not free-flowing and was cohesive in nature. SEM images (Figure 3) showed that porous aggregates were formed for PLH microparticles in the size range 20−100 μm, which readily redisperse in aqueous solution to individual microparticles with an approximate particle size of 5 μm. The dry state aggregation of PLH microparticles was evident for both PLGA(+) and PLGA(−) nanoparticles, despite differences in surface morphology, and was therefore independent of nanoparticle charge. It was hypothesized that aggregation was influenced by the high temperatures and shear forces of spray drying, disrupting the structure of lipid droplets and PLGA nanoparticles resulting in the formation of cohesive microparticles.

Unlike other robust carrier materials commonly used in spray drying, PLGA has a low glass transition temperature which limits the flowability of the formed powder and can introduce difficulties maintaining the integrity of PLGA nanoparticles during this process.[36] Carbohydrates, such as mannitol, act as bulking agents and provide protection to PLGA nanoparticles from shear forces and high temperatures during spray drying,[37,38] while reducing the overall water content in the dry material.[36] As a result, mannitol was administered during

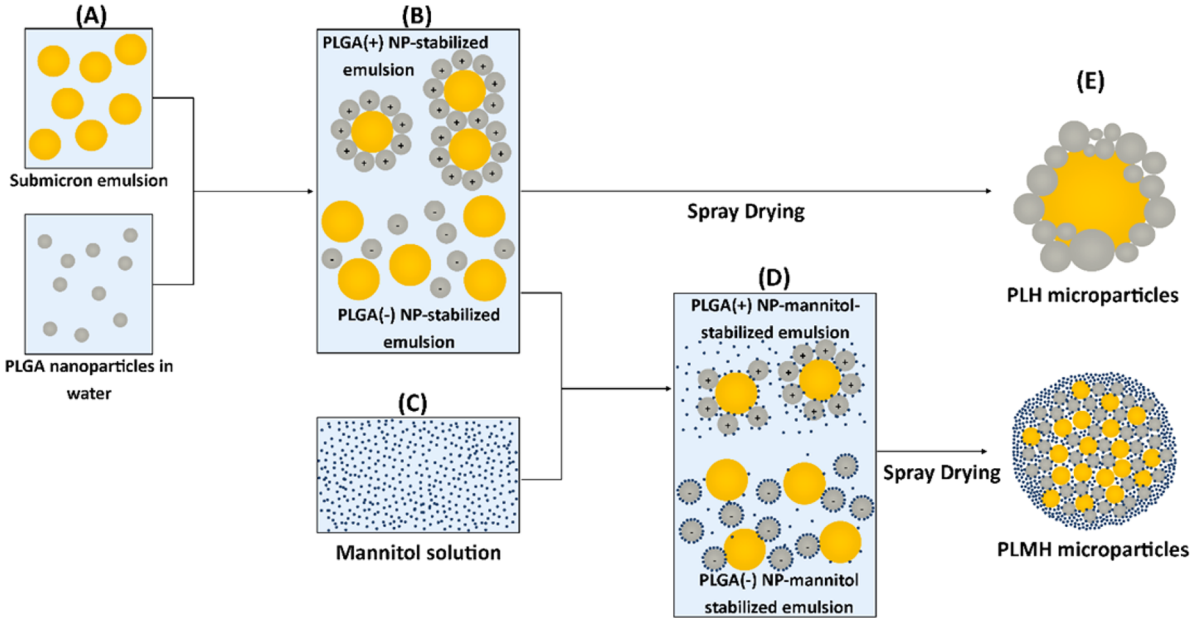

Figure 2. Schematic representation of the two-step fabrication process for PLGA−lipid hybrid (PLH) and PLGA−lipid−mannitol hybrid (PLMH) microparticles using PLGA nanoparticle (NP) stabilized emulsions as precursors. (A) Homogenized lipid droplets in water and PLGA nanoparticles were dispersed in water using sonication. (B) PLGA nanoparticle stabilized emulsions formed. (C) Mannitol solution was dispersed in stabilized emulsions using sonication. (D) PLGA−mannitol-stabilized emulsions formed. (E) Spray dried stabilized emulsions formed PLH and PLMH microparticles in the absence and presence of mannitol, respectively.

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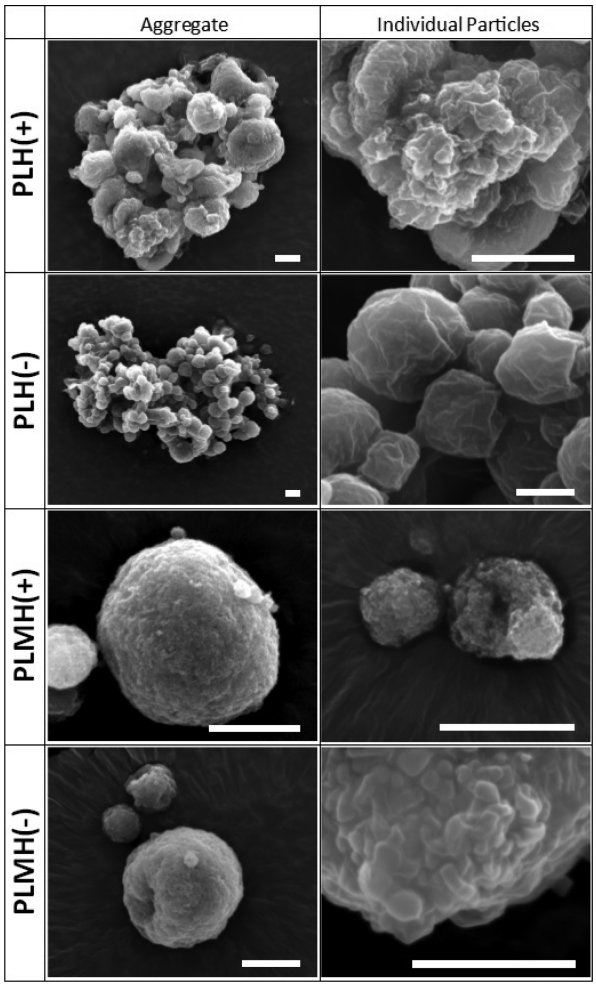

Figure 3. SEM images of PLH microparticles with varying particle charges and cryoprotectant contents, highlighting the aggregation behavior and surface morphology of each particle. Scale bars represent 2 μm.

the preparation process (step C, Figure 2) to investigate the effect of a carbohydrate cryoprotectant on the nanoparticle integrity and microparticle aggregation during the water removal step. The ζ potential, and particle size, increased for PLGA–lipid–mannitol hybrid (PLMH) microparticles compared to PLH microparticles (Table 1), yielding a pK_a between 10 and 11 for PLMH(+) microparticles and between 6 and 7 for PLMH(−) microparticles (Supporting Information, Figure S2). Both positively and negatively charged nanoparticle-stabilized emulsions formed free-flowing powders when spray dried with an aqueous solution of mannitol, as evidenced by minimal particle aggregation in SEM images (Figure 3). The surface morphologies of PLMH(+) and PLMH(−) microparticles were equivalent, and both consisted of rough particles in appearance in the size range of 2−5 μm. Thus, the surface morphology was independent of the ζ potential of the primary nanoparticles, when mannitol was used in the preparation process.

The difference in morphology and particle aggregation between PLH and PLMH microparticles was attributed to the addition of mannitol during the water removal process. SEM images demonstrated a smoother spherical morphology for PLH microparticles compared to PLMH microparticles. Confocal fluorescence microscopy was used to provide further insights into the differences in morphology and structure for particles in the presence and absence of mannitol. Obtained images showed PLGA nanoparticles accumulated at the particle surface for PLH(−) microparticles, coating large lipid droplets in the particle core (Figure 4). It was evident that the lipid

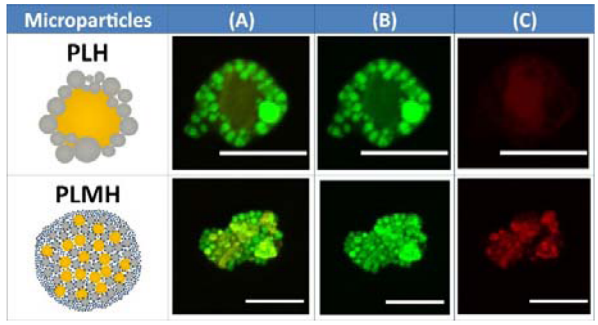

Figure 4. Confocal fluorescence images of PLH(−) microparticles and PLMH(−) microparticles. (A) Overlay; (B) λ = 488 nm (PLGA component); (C) λ = 561 nm (lipid component). Scale bars represent 5 μm.

droplets did not maintain structural integrity in the absence of mannitol and underwent aggregation during spray drying. In comparison, confocal fluorescence images of PLMH microparticles showed mannitol increased the ability for the self-assembly of PLGA nanoparticles from the continuous phase to the droplet interface during spray drying, forming a three-dimensional porous structure whereby submicrometer lipid droplets were encapsulated within a PLGA nanoparticle matrix, similarly to the previously formed SLH microparticles by Tan et al.[18]

Redispersion Properties of Hybrid Microparticles in Biorelevant Media. The ability of hybrid microparticles to redisperse and disintegrate into smaller heterogeneous aggregations of PLGA nanoparticles and lipid droplets in digestion media was investigated by monitoring the change in particle size over a 60 min period (Figure 5). Simulated gastrointestinal media were used during the redispersion of hybrid particles to mimic the potential mechanism of particle breakdown in the gastrointestinal tract. In all cases, anionic microparticles demonstrated an enhanced ability to redisperse

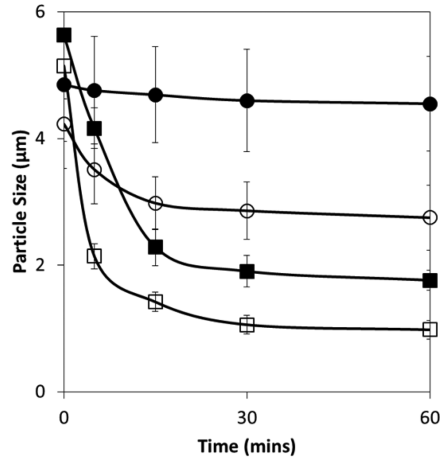

Figure 5. Particle size as a function of time for (●) PLH(+), (○) PLH(−), (■) PLMH(+), and (□) PLMH(−) microparticles, upon redispersion in fasted state digestion medium.

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into smaller particles, compared to cationic microparticles. That is, the average particle size of PLH(+) microparticles was unaltered (4.85 ± 0.9 to 4.55 ± 0.75 μm) compared to 4.23 ± 0.7 to 2.75 ± 0.51 μm for PLH(−) microparticles, and the average particle size of PLMH(+) microparticles decreased from 5.63 ± 1.2 to 1.76 ± 0.3 μm compared to 5.15 ± 1.0 to 0.98 ± 0.2 μm for PLMH(−) microparticles. The difference in degrees of disintegration for particles with different surface charge was attributed to the type of electrostatic interactions between the PLGA nanoparticles and lipid droplets. As previously shown in Figure 1, PLGA(+) nanoparticles coagulate with negatively charged lipid droplets in the wet phase through electrostatic attraction. This interaction increases the stability of the hybrid microparticle structure, reducing its redispersion in digestion media compared to microparticles composed of PLGA(−) nanoparticles and lipid droplets. Simovic et al.[20] demonstrated an equivalent electrostatic interaction between porous silica particles and lipid droplets in SLH microparticles, whereby particles with oppositely charged silica and lipid components also showed greater redispersibility in aqueous environments.

A noticeable difference in the rate and extent of redispersion of microparticles was observed between particles with and without mannitol. The minimal change in particle size for PLH(+) and PLH(−) microparticles confirms the hypothesis that the high temperatures and shear stress of the water removal process, in the absence of mannitol, induced aggregation of lipid droplets within the core of the hybrid particles, as observed by confocal fluorescence microscopy. This inhibited the redispersion of microparticles into individual submicrometer lipid droplets and PLGA nanoparticles. Mannitol, however, enhanced the redistribution properties of hybrid microparticles as evidenced by a 4- to 5-fold reduction in particle sizes for PLMH(+) and PLMH(−) microparticles, respectively. This coincides with previous findings by Jensen et al.,[39] who demonstrated the importance of mannitol on the redispersibility of a spray dried PLGA nanoparticle matrix. Thus, mannitol protected PLGA nanoparticle stabilized lipid droplets from the harsh spray drying conditions resulting in a three-dimensional structure which readily redispersed to PLGA nanoparticles and lipid droplets when added to digestion media.

In Vitro Lipid Digestion Studies. *Influence of PLGA Nanoparticles on Digestion of Lipid Droplets.* The effect of PLGA nanoparticles on pancreatic lipase action was examined by in vitro lipid digestion, under simulated intestinal conditions, in stabilized emulsion systems with varying concentrations of PLGA(+) and PLGA(−) nanoparticles (Figure 6). Emulsification of lipid facilitates a greater exposure of the lipid substrate to lipase during the early phase of digestion,[40] due to an increase in surface area of lipid.[41] Consequently, fast initial lipolysis kinetics was observed for the bare submicrometer emulsion. As digestion proceeded, the rate of digestion was promptly inhibited due to the accumulation of interfacially active digestion products and lecithin on the emulsion droplet interface.[24] At high concentrations, or as the mean emulsion droplet size decreases, lecithin interferes with lipase adsorption by competing with the enzyme for sites on the oil−water interface.[42] In addition to this, at insufficient bile salt concentrations, digestion products (free fatty acids and monoglycerides), adsorb to the oil−water interface due to their amphiphilic structure.[43] The rapid decrease in emulsion droplet size and release of digestion products leads to an

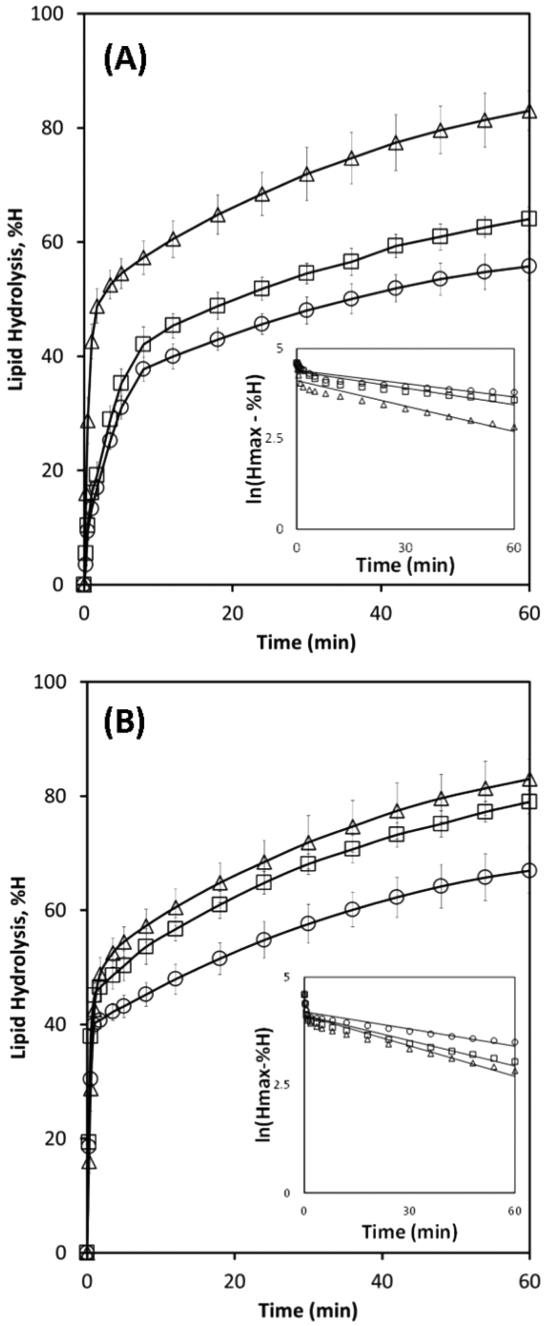

Figure 6. Lipid hydrolysis as a function of time for submicrometer MCT emulsions stabilized by (A) PLGA(+) nanoparticles and (B) PLGA(−) nanoparticles. PLGA nanoparticles were added to the aqueous phase at (△) 0, (□) 10, and (○) 50 wt %, relative to the lipid content in the emulsion systems. (inset) Pseudo-first-order fit.

inhibition of enzymatic degradation due to a saturation of the oil−water interface, evident at approximately 60% digestion.

Pseudo-first-order kinetics were used to describe the lipid digestion of all stabilized emulsion systems (eq 1). The first-order rate constants, k, were determined for each lipid system by fitting a curve to $\ln(H_{max} - \%H)$ versus time and are given in Table 2. First-order kinetics do not precisely describe the entire digestion profile of a submicrometer emulsion (Figure 6, inset) due to the interference effect of digestion products causing a sharp decrease in reaction rate.[24] Consequently, the first-order rate constants for the bare and stabilized emulsions

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Table 2. Kinetic Analysis Data for the Lipolysis of MCT Lipid Systems Using Pseudo-First-Order Kinetics

lipid formulation	lipid hydrolysis rate const, k ($min^{-1} \times 10^{-2}$)	extent lipid hydrolysis after 3 h, H_{max}
coarse emulsion	1.91 (R^2 = 0.98)	94 ± 0.6
submicrometer emulsion	30.5 (R^2 = 0.79)	94 ± 3.3
PLGA(+) NP stabilized emulsion (10 wt %)	9.40 (R^2 = 0.94)	64 ± 2.1
PLGA(+) NP stabilized emulsion (50 wt %)	7.60 (R^2 = 0.91)	56 ± 2.4
PLGA(−) NP stabilized emulsion (10 wt %)	19.2 (R^2 = 0.81)	79 ± 2.9
PLGA(−) NP stabilized emulsion (50 wt %)	14.5 (R^2 = 0.80)	67 ± 4.0
PLH(+) microparticles	1.70 (R^2 = 0.96)	97 ± 1.2
PLH(−) microparticles	2.43 (R^2 = 0.96)	98 ± 1.9
PLMH(+) microparticles	3.69 (R^2 = 0.96)	100 ± 0
PLMH(−) microparticles	4.87 (R^2 = 0.96)	100 ± 0

only describe the initial kinetics of digestion (e.g., the k value of 0.305 for the submicrometer emulsion only accurately describes the first 2 min of digestion).

Digestion kinetics were inhibited in the presence of PLGA nanoparticles at all concentrations compared to a bare submicrometer emulsion. This was demonstrated by a decrease in rate constants from 0.305 min^{-1} for the bare submicrometer emulsion to 0.094 and 0.192 min^{-1} for PLGA(+) and PLGA(−) nanoparticle stabilized emulsions, respectively, at 10 wt % nanoparticle concentration. It is hypothesized that PLGA nanoparticles inhibit lipase activity via one or more of the following mechanisms: (i) sterically hindering lipase by physically shielding the droplet interface,[44] (ii) providing a hydrophobic interface for lipase adsorption via hydrophobic interactions, thus competitively inhibiting lipolysis,[45] and/or (iii) reducing the available surface area of lipid.[40]

For both PLGA(+) and PLGA(−) nanoparticle stabilized emulsions, as the concentration of nanoparticles increased from 10 to 50 wt %, the rate and extent of digestion decreased due to increased physical shielding and steric hindrance. However, the degree of inhibition of lipase was significantly greater for PLGA(+) nanoparticles than in PLGA(−) nanoparticles due to the difference in nanoparticle charge. The overall extents of lipolysis for 10 and 50 wt % PLGA(−) nanoparticle stabilized emulsions were 64.0 ± 3.6 and 55.8 ± 2.9%, respectively, compared to 78.9 ± 5.6 and 66.9 ± 5.1% for 10 and 50 wt % PLGA(+) nanoparticle stabilized emulsions, respectively. The first-order rate constants were also significantly reduced when submicrometer emulsion droplets were stabilized with PLGA-(+) nanoparticles (0.076 min^{-1} at 50 wt % nanoparticle concentration), compared to PLGA(−) nanoparticles (0.145 min^{-1} at 50 wt % nanoparticle concentration). This coincided with the enhanced ability for cationic nanoparticles to stabilize and coagulate with anionic emulsion droplets, at pH 7.5, due to the electrostatic attraction between both interfaces (Figure 1). Consequently, the extent of droplet coating by the nanoparticles at the lipid-in-water interface was significantly greater for PLGA(+) nanoparticles, reducing the rate of lipase adsorption to the emulsion droplets. A similar effect was observed by Tan et al.,[21] whereby the steric hindrance on lipase by silica nanoparticles increased as nanoparticle concentration increased and was greatest for nanoparticles that were oppositely charged to the lipid droplets.

PLGA(−) nanoparticles carry a small negative charge at neutral pH. Consequently, electrostatic repulsive interactions between nanoparticles and emulsion droplets restrict the number of nanoparticles that can weakly adsorb to the droplet interface through hydrophobic interactions. The lipolysis rate constants were therefore greater for PLGA(−) nanoparticle stabilized emulsions compared to PLGA(+) nanoparticle stabilized emulsions due to the ability for lipase to gain access to the lipid interface. However, as lipid digestion proceeded for PLGA(−) nanoparticle stabilized emulsions, the emulsion droplets decreased in size, increasing the shielding effect of the PLGA(−) nanoparticles and restricting the access of lipase to the oil−water interface, leading to reduced extents of lipolysis over a 60 min period, compared to the submicrometer emulsion.

Spray Dried PLGA−Lipid Hybrid Microparticles. Figure 7 depicts the percent of lipid hydrolysis as a function of time over

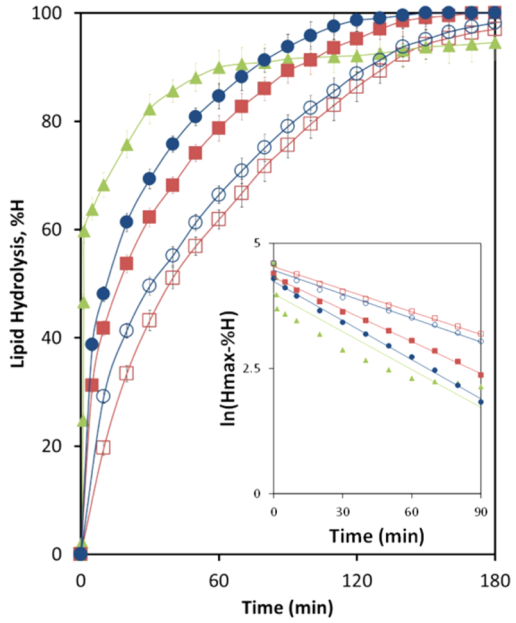

Figure 7. Lipase-mediated digestion kinetics for MCT in (red ○) PLH(+) microparticles, (blue ○) PLH(−) microparticles, (red ■) PLMH(+) microparticles, (blue ■) PLMH(−) microparticles, and (green ▲) a submicrometer emulsion, under fasted state conditions over a 3-h digestion period. (inset) Pseudo-first-order fit.

an extended 3 h period for the four developed hybrid microparticles (PLH(+), PLH(−), PLMH(+), and PLMH(−) microparticles) compared to a submicrometer MCT emulsion stabilized with lecithin. Lipolysis of all four hybrid microparticles occurred at a slower initial rate due to the steric hindrance of PLGA on enzyme adsorption. However, the overall extents of digestion for all hybrid microparticles were equivalent to the emulsion (100% for PLMH(+) and PLMH(−) microparticles versus 94.5 ± 3.3% for submicrometer emulsion). Both the submicrometer emulsion and lipid droplets within the dry microparticles were stabilized with lecithin. The inhibitory effect of lecithin and digestion products was clearly evident for the digestion of the submicrometer emulsion. In comparison, a mechanism must exist for PLH and PLMH microparticles whereby lecithin and the released digestion products were removed from the lipid interface, facilitating enhanced lipase adsorption which resulted in

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complete digestion. It is hypothesized that the electrostatic interaction between the PLGA nanoparticles and negatively charged digestion products reduces the number of amphiphilic components that adsorb to the oil−water interface, and increases the level of vesicular and micellar structure formation.[24]

Interestingly, hydrolysis of lipid encapsulated within PLH and PLMH microparticles was well described by pseudo-first-order kinetics (Table 2). Whereas, the k value of 0.305 min^{-1} only describes the initial rate of digestion for submicrometer emulsion droplets, which is consequently significantly greater than those for the PLH microparticles. In contrast to this, digestion of lipid within silica−lipid hybrid microparticles, prepared by spray drying silica nanoparticle stabilized emulsion droplets, demonstrated triphasic pseudo-first-order kinetics and thus required three first-order rate constants to precisely describe the data.[22] Digestion kinetics were enhanced in SLH microparticles compared to a submicrometer emulsion due to the increased surface area of lipid and the hydrophilic silica matrix facilitating the interfacial activation of lipase. The difference in digestion kinetics between the hybrid systems of silica and PLGA indicates that the change in surface chemistry and larger particle size of PLGA nanoparticles compared to porous silica alters the interaction between lipase and the lipid interface. It is hypothesized that lipase adsorbs to the mostly hydrophobic PLGA surface in its inactive, closed-lid conformation through hydrophobic interactions, reducing the number of enzyme molecules available for catalysis and thereby reducing the lipid digestion rate.[46] Furthermore, the increased particle size of PLGA nanoparticles compared to silica nanoparticles increases the steric hindrance of enzyme molecules to the lipid interface. The difference in lipase-mediated digestion kinetics between PLH and SLH microparticles is predicted to alter the release profiles of bioactive compounds encapsulated within the lipid component of PLH microparticles and potentially result in sustained absorption into the blood, a desirable characteristic for many poorly water-soluble drugs. That is, the rate of drug solubilization within a lipid-based carrier, and thereby absorption, is altered by the digestibility of lipid by pancreatic lipase.[3] Thus, more sustained lipase-mediated digestion kinetics in PLH microparticles is likely to lead to sustained drug solubilization in the gastrointestinal tract.

Lipid hydrolysis of all dry hybrid microparticles demonstrated sustained digestion kinetics compared to PLGA nanoparticle stabilized emulsions, indicating there was a greater level of physical shielding of lipase by PLGA in the dry phase compared to the wet phase. The overall extent of lipolysis for PLH(+) microparticles was 61.8 ± 3.7% over a 60 min digestion period, which was consistent with the enzymatic degradation of lipid for a 10 wt % PLGA(+) stabilized emulsion. However, the pseudo-first-order rate constant for PLH(+) microparticles was 0.017 min^{-1} compared to 0.094 min^{-1} for the PLGA(+) nanoparticle stabilized emulsion at this concentration. Hence, the rate of lipid hydrolysis was significantly slower for PLH microparticles, but the overall extent of digestion was equal to a PLGA-stabilized emulsion. Slow initial digestion kinetics suggests the slow transport and adsorption of the enzyme from the aqueous continuous phase before becoming catalytically active at the interface.[21] However, equivalent extents of digestion suggested the microstructure facilitated sustained digestion and a reduced interference effect of digestion products and PLGA nanoparticles on lipase adsorption.[47]

The digestion kinetics were enhanced when PLGA(−) nanoparticles were used to prepare hybrid microparticles, in both the presence and absence of mannitol, when compared with PLGA(+) nanoparticles. That is, the pseudo-first-order rate constant increased from 0.017 and 0.037 min^{-1} for positively charged PLH(+) and PLMH(+) microparticles, respectively, compared to 0.024 and 0.049 min^{-1} for negatively charged PLH(−) and PLMH(−) microparticles, respectively (Table 2). This was consistent with previous studies that have demonstrated the effect of surface charge on digestion of conventional emulsion droplets.[42,48] Positively charged lipid droplets coagulate due to electrostatic interactions between the positively charged droplet interface and negatively charged digestion products that adsorb to the interface, reducing the accessible surface area for lipase adsorption.[41] This was further supported by reduced microparticle redispersibility when PLGA(+) nanoparticles were used to prepare hybrid microparticles (Figure 5). Since lipid digestion is controlled by the ability for lipase to access the lipid interface,[49,50] it is hypothesized an increase in hybrid particle disintegration increased the accessible surface area of lipid, which enhanced lipase activity in PLH(−) and PLMH(−) microparticles.

The rate and extent of lipolysis increased when mannitol was incorporated in the hybrid particles. Confocal fluorescence microscopy was used to investigate the influence of mannitol on lipid digestion and deconvolute the relationship between microparticle redispersibility and disintegration on lipase activity for PLH(−) and PLMH(−) microparticles. Confocal fluorescence images were taken at three time points (0, 5, and 60 min) during dispersion to demonstrate the lipid environment accessible by lipase during digestion (Figure 8). As digestion proceeded, PLMH(−) microparticles redispersed to smaller heterogeneous aggregates of PLGA(−) nanoparticles and lipid droplets (≤1 μm at 60 min), whereas PLH(−) microparticles maintained particles in the micrometer size range (Figure 8, 60 min), which was consistent with Figure 5. The formation of small aggregates of PLGA nanoparticles and emulsion droplets from PLMH microparticles increased the interfacial surface area of lipid, whereas the inability for PLH microparticles to redisperse to primary particles and droplets due to the microcapsule-like structure restricted lipase adsorption, producing more sustained digestion kinetics that were similar to a coarse emulsion. This was evident in confocal fluorescence images as the degree of lipid content decreased in PLMH(−) microparticles significantly more over time compared to PLH(−) microparticles.

While previously prepared lipid coated PLGA particles have demonstrated promising drug delivery applications,[17] the novel microstructure of PLH and three-dimensional matrix structure of PLMH microparticles synthesized in this study present architectures with promising delivery characteristics that remove stability concerns common in lipid coated PLGA particles. By controlling lipase action through structural properties, it is hypothesized that these novel microparticles can facilitate controlled drug release and absorption from the lipid component, in comparison to the common burst release mechanism of lipid coated PLGA particles. Sustained lipid digestion is predicted to enhance lipophilic drug solubilization and reduce the potential for drug precipitation for a wide range of poorly water-soluble drugs. In addition to this, PLH microparticles do not share the same biocompatibility and

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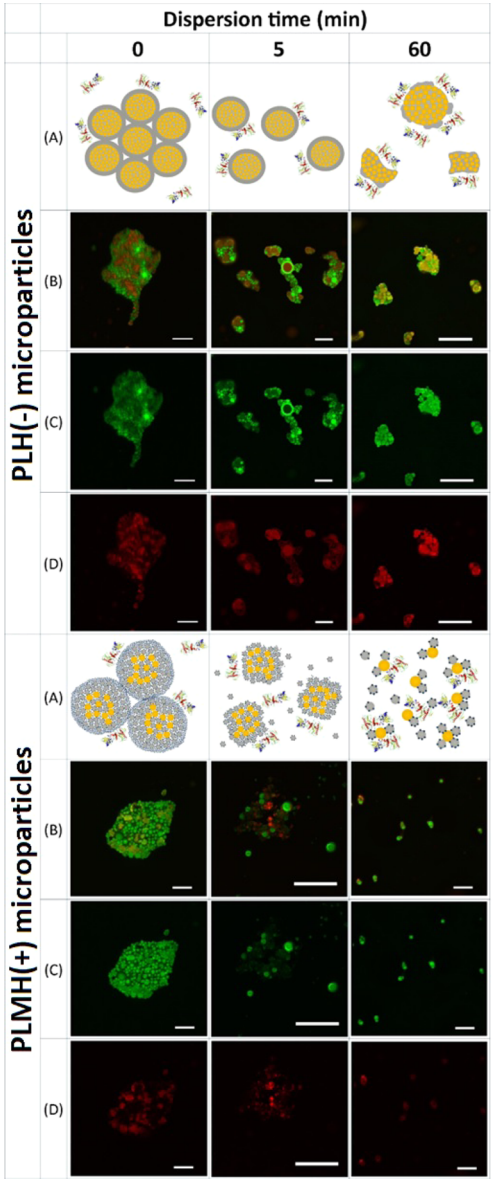

Figure 8. Confocal fluorescence cross-section images and schematic representations of PLH(−) and PLMH(−) microparticles at 0, 5, and 60 min redispersion in digestion media. (A) Schematic representations: PLGA = gray, lipid = yellow droplets, mannitol = blue. CLSM images: (B) overlay, (C) λ = 488 nm (PLGA component), and (D) λ = 561 nm (lipid component). Scale bars = 3 μm.

toxicity concerns associated with the previously prepared SLH microparticles, due to the high biocompatibility of PLGA nanoparticles. We propose the microparticles presented will act as a novel drug nanocarrier with a wide range of therapeutic applications, such as combinational therapy, oral delivery of lipophilic drugs, and localized delivery.

CONCLUSIONS

Novel PLGA−lipid hybrid (PLH) microparticles were prepared for the first time by spray drying PLGA nanoparticle stabilized emulsions. Particle structure and redispersibility in aqueous media were controlled by the nanoparticle stabilizer and cryoprotectant content. The rate of redispersion was greatest for negatively charged microparticles in the presence of mannitol. Sustained lipid digestion was evident in all PLH systems, whereby the initial digestion rate was reduced, but the overall extent of digestion over 60 min was equivalent to a stabilized submicrometer emulsion, demonstrating the bioactivity of the prepared microparticles. Lipid digestion kinetics were tailored and controlled by introducing mannitol to the PLH microparticles and altering the surface charge; as the surface charge decreased, the rate and extent of digestion increased. This suggests the degree of physical shielding by PLGA was greater in hybrid microparticles but the structure facilitated a mechanism that prevented competitive inhibition from surface active digestion products and PLGA nanoparticles. PLH systems demonstrate significant potential for drug delivery.

ASSOCIATED CONTENT

Supporting Information

Physicochemical properties of PLGA nanoparticles, example of the TGA evaluation employed to determine lipid content, and ζ potential as a function of pH for synthesized PLGA nanoparticles, submicrometer emulsion droplets, and hybrid microparticles. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b05068.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Raemdonck, K.; Braeckmans, K.; Demeester, J.; De Smedt, S. C. Merging the Best of Both Worlds: Hybrid Lipid-Enveloped Matrix Nanocomposites in Drug Delivery. *Chem. Soc. Rev.* **2014**, *43* (1), 444−472.

(2) Bajaj, H.; Bisht, S.; Yadav, M.; Singh, V.; Singh, M. Self Emulsifying Drug Delivery System: An Approach to Enhance Bioavailability. *Int. J. Pharm. Res. Dev.* **2011**, *3* (1), 59−75.

(3) Porter, C. J. H.; Pouton, C. W.; Cuine, J. F.; Charman, W. N. Enhancing Intestinal Drug Solubilisation Using Lipid-Based Delivery Systems. *Adv. Drug Delivery Rev.* **2008**, *60* (6), 673−691.

(4) Jannin, V.; Musakhanian, J.; Marchaud, D. Approaches for the Development of Solid and Semi-Solid Lipid-Based Formulations. *Adv. Drug Delivery Rev.* **2008**, *60* (6), 734−746.

(5) Pouton, C. W.; Porter, C. J. H. Formulation of Lipid-Based Delivery Systems for Oral Administration: Materials, Methods and Strategies. *Adv. Drug Delivery Rev.* **2008**, *60* (6), 625−637.

(6) Simovic, S.; Barnes, T. J.; Tan, A.; Prestidge, C. A. Assembling Nanoparticle Coatings to Improve the Drug Delivery Performance of Lipid Based Colloids. *Nanoscale* **2012**, *4* (4), 1220−1230.

(7) Pillai, O.; Panchagnula, R. Polymers in Drug Delivery. *Curr. Opin. Chem. Biol.* **2001**, *5*, 447−451.

(8) Italia, J. L.; Yahya, M. M.; Singh, D.; Ravi Kumar, M. N. V. Biodegredable Nanoparticles Improve Oral Bioavailability of Ampho-

DOI: 10.1021/acsami.5b05068
ACS Appl. Mater. Interfaces 2015, 7, 17460−17470

tericin B and Show Reduced Nephrotoxicity Compared to Intravenous Fungizone. *Pharm. Res.* **2009**, *26* (6), 1324−1331.
(9) Kumari, A.; Yadav, S. K.; Yadav, S. C. Biodegradable Polymeric Nanoparticles Based Drug Delivery Systems. *Colloids Surf., B* **2010**, *75* (1), 1−18.
(10) Kumbhar, D. D.; Pokharkar, V. B. Physicochemical Investigations on an Engineered Lipid−Polymer Hybrid Nanoparticle Containing a Model Hydrophilic Active, Zidovudine. *Colloids Surf., A* **2013**, *436*, 714−725.
(11) Liu, Y.; Pan, J.; Feng, S.-S. Nanoparticles of Lipid Monolayer Shell and Biodegradable Polymer Core for Controlled Release of Paclitaxel: Effects of Surfactants on Particles Size, Characteristics and In Vitro Performance. *Int. J. Pharm.* **2010**, *395* (1), 243−250.
(12) Mandal, B.; Bhattacharjee, H.; Mittal, N.; Sah, H.; Balabathula, P.; Thoma, L. A.; Wood, G. C. Core−Shell-Type Lipid−Polymer Hybrid Nanoparticles as a Drug Delivery Platform. *Nanomedicine* **2013**, *9* (4), 474−491.
(13) Thevenot, J.; Troutier, A.-L.; Putaux, J.-L.; Delair, T.; Ladavière, C. Effect of the Polymer Nature on the Structural Organization of Lipid/Polymer Particle Assemblies. *J. Phys. Chem. B* **2008**, *112* (44), 13812−13822.
(14) Thevenot, J.; Troutier, A.-L.; David, L.; Delair, T.; Ladavière, C. Steric Stabilization of Lipid/Polymer Particle Assemblies by Poly-(ethylene glycol)-Lipids. *Biomacromolecules* **2007**, *8* (11), 3651.
(15) Troutier, A.-L.; Delair, T.; Pichot, C.; Ladavière, C. Physicochemical and Interfacial Investigation of Lipid/Polymer Particle Assemblies. *Langmuir* **2005**, *21* (4), 1305−1313.
(16) Bala, I.; Hariharan, S.; Kumar, M. PLGA Nanoparticles in Drug Delivery: The State of the Art. *Crit. Rev. Ther. Drug Carrier Syst.* **2004**, *21* (5), 387−422.
(17) Simovic, S.; Heard, P.; Hui, H.; Song, Y.; Peddie, F.; Davey, A. K.; Lewis, A.; Rades, T.; Prestidge, C. A. Dry Hybrid Lipid-Silica Microcapsules Engineered from Submicron Lipid Droplets and Nanoparticles as a Novel Delivery System for Poorly Soluble Drugs. *Mol. Pharmaceutics* **2009**, *6* (3), 861−872.
(18) Tan, A.; Simovic, S.; Davey, A. K.; Rades, T.; Prestidge, C. A. Silica-Lipid Hybrid (SLH) Microcapsules: A Novel Oral Delivery System for Poorly Soluble Drugs. *J. Controlled Release* **2009**, *134*, 62−70.
(19) Tan, A.; Martin, A.; Nguyen, T.-H.; Boyd, B. J.; Prestidge, C. A. Hybrid Nanomaterials That Mimic the Food Effect: Controlling Enzymatic Digestion for Enhanced Oral Drug Absorption. *Angew. Chem., Int. Ed.* **2012**, *51* (22), 5475−5479.
(20) Simovic, S.; Heard, P.; Prestidge, C. A. Hybrid Lipid-Silica Microcapsules Engineered by Phase Coacervation of Pickering Emulsions to Enhance Lipid Hydrolysis. *Phys. Chem. Chem. Phys.* **2010**, *12*, 7162−7170.
(21) Tan, A.; Simovic, S.; Davey, A. K.; Rades, T.; Boyd, B. J.; Prestidge, C. A. Silica Nanoparticles To Control the Lipase-Mediated Digestion of Lipid-Based Oral Delivery Systems. *Mol. Pharmaceutics* **2010**, *7* (2), 522−532.
(22) Tan, A.; Davey, A. K.; Prestidge, C. A. Silica-Lipid Hybrid (SLH) *versus* Non-lipid Formulations for Optimising the Dose-Dependent Oral Absorption of Celecoxib. *Pharm. Res.* **2011**, *28*, 2273−2287.
(23) Simovic, S.; Hui, H.; Song, Y.; Davey, A. K.; Rades, T.; Prestidge, C. A. An Oral Delivery System for Indomethacin Engineered from Cationic Lipid Emulsions and Silica Nanoparticles. *J. Controlled Release* **2010**, *143*, 367−373.
(24) Joyce, P.; Tan, A.; Whitby, C. P.; Prestidge, C. A. The Role of Porous Nanostructure in Controlling Lipase-Mediated Digestion of Lipid Loaded Into Silica Particles. *Langmuir* **2014**, *30* (10), 2779−2788.
(25) Yu, T.; Greish, K.; Mcgill, L. D.; Ray, A.; Ghandehari, H. Influence of Geometry, Porosity, and Surface Characteristics of Silica Nanoparticles on Acute Toxicity: Their Vasculature Effect and Tolerance Threshold. *ACS Nano* **2012**, *6* (3), 2289−2301.
(26) Whitby, C. P.; Lim, L. H.; Ghouchi Eskandar, N.; Simovic, S.; Prestidge, C. A. Poly(Lactic-co-Glycolic Acid) as a Particulate Emulsifier. *J. Colloid Interface Sci.* **2012**, *375* (1), 142−147.
(27) Hariharan, S.; Bhardwaj, V.; Bala, I.; Sitterberg, J.; Bakowsky, U.; Ravi Kumar, M. N. V. Design of Estradiol Loaded PLGA Nanoparticulate Formulations: a Potential Oral Delivery System for Hormone Therapy. *Pharm. Res.* **2006**, *23* (1), 184−195.
(28) Peltonen, L.; Koistinen, P.; Karjalainen, M.; Häkkinen, A.; Hirvonen, J. The Effect of Cosolvents on the Formulation of Nanoparticles from Low-Molecular-Weight Poly(l)lactide. *AAPS PharmSciTech* **2002**, *3* (4), 52−58.
(29) Song, K. C.; Lee, H. S.; Choung, I. Y.; Cho, K. I.; Ahn, Y.; Choi, E. J. The Effect of Type of Organic Phase Solvents on the Particle Size of Poly(d,l-lactide-co-glycolide) Nanoparticles. *Colloids Surf., A* **2006**, *276* (1), 162−167.
(30) Jang, D.-J.; Jeong, E. J.; Lee, H.-M.; Kim, B.-C.; Kim, C.-K.; Lim, S.-J. Improvement of Bioavailability and Photostability of Amlodipine Using Redispersible Dry Emulsion. *Eur. J. Pharm. Sci.* **2006**, *28* (5), 405−411.
(31) Sek, L.; Porter, C. J. H.; Kaukonen, A. M.; Charman, W. N. Evaluation of the In-Vitro Digestion Profiles of Long and Medium Chain Glycerides and the Phase Behaviour of their Lipolytic Products. *J. Pharm. Pharmacol.* **2002**, *54* (1), 29−41.
(32) Astete, C. E.; Sabliov, C. M. Synthesis and Characterization of PLGA Nanoparticles. *J. Biomater. Sci., Polym. Ed.* **2006**, *17* (3), 247−289.
(33) Sahana, D. K.; Mittal, G.; Bhardwaj, V.; Ravi Kumar, M. N. V. PLGA Nanoparticles for Oral Delivery of Hydrophobic Drugs: Influence of Organic Solvent on Nanoparticles Formation and Release Behavior *In Vitro* and *In Vivo* Using Estradiol as a Model Drug. *J. Pharm. Sci.* **2008**, *97* (4), 1530−1542.
(34) Horozov, T. S.; Binks, B. P. Particle-Stabilized Emulsions: A Bilayer or a Bridging Monolayer? *Angew. Chem., Int. Ed.* **2006**, *45* (5), 773−776.
(35) Eskandar, N. G.; Simovic, S.; Prestidge, C. A. Synergistic Effect of Silica Nanoparticles and Charged Surfactants in the Formation and Stability of Submicron Oil-in-Water Emulsions. *Phys. Chem. Chem. Phys.* **2007**, *9*, 6426−6434.
(36) Jensen, D. M. K.; Cun, D.; Maltesen, M. J.; Frokjaer, S.; Nielsen, H. M.; Foged, C. Spray Drying of siRNA-Containing PLGA Nanoparticles Intended for Inhalation. *J. Controlled Release* **2010**, *142* (1), 138−145.
(37) Tomoda, K.; Ohkoshi, T.; Kawai, Y.; Nishiwaki, M.; Nakajima, T.; Makino, K. Preparation and Properties of Inhalable Nanocomposite Particles: Effects of the Temperature at a Spray-Dryer Inlet upon the Properties of Particles. *Colloids Surf., B* **2008**, *61* (2), 138−144.
(38) Takashima, Y.; Saito, R.; Nakajima, A.; Oda, M.; Kimura, A.; Kanazawa, T.; Okada, H. Spray-Drying Preparation of Microparticles Containing Cationic PLGA Nanospheres as Gene Carriers for Avoiding Aggregation of Nanospheres. *Int. J. Pharm.* **2007**, *343* (1−2), 262−269.
(39) Jensen, D. K.; Jensen, L. B.; Koocheki, S.; Bengtson, L.; Cun, D.; Nielsen, H. M.; Foged, C. Design of an Inhalable Dry Powder Formulation of DOTAP-Modified PLGA Nanoparticles Loaded with siRNA. *J. Controlled Release* **2012**, *157* (1), 141−148.
(40) Armand, M.; Borel, P.; Ythier, P.; Dutot, G.; Melin, C.; Senft, M.; Lafont, H.; Lairon, D. Effects of Droplet Size, Triacylglycerol Composition, and Calcium on the Hydrolysis of Complex Emulsions by Pancreatic Lipase: An In Vitro Study. *J. Nutr. Biochem.* **1992**, *3* (7), 333−341.
(41) Chu, B.-S.; Rich, G. T.; Ridout, M. J.; Faulks, R. M.; Wickham, M. S. J.; Wilde, P. J. Modulating Pancreatic Lipase Activity with Galactolipids: Effects of Emulsion Interfacial Composition. *Langmuir* **2009**, *25* (16), 9352−9360.
(42) Vinarov, Z.; Tcholakova, S.; Damyanova, B.; Atanasov, Y.; Denkov, N. D.; Stoyanov, S. D.; Pelan, E.; Lips, A. Effects of Emulsifier Charge and Concentration on Pancreatic Lipolysis: 2. Interplay of Emulsifiers and Biles. *Langmuir* **2012**, *28* (33), 12140−12150.

DOI: 10.1021/acsami.5b05068
ACS Appl. Mater. Interfaces 2015, 7, 17460−17470

(43) Reis, P.; Holmberg, K.; Miller, R.; Kragel, J.; Grigoriev, D. O.; Leser, M. E.; Watzke, H. J. Competition Between Lipases and Monoglycerides at Interfaces. *Langmuir* **2008**, *24* (14), 7400−7407.
(44) Singh, H.; Ye, A.; Horne, D. Structuring Food Emulsions in the Gastrointestinal Tract to Modify Lipid Digestion. *Prog. Lipid Res.* **2009**, *48* (2), 92−100.
(45) Reis, P.; Holmberg, K.; Watzke, H.; Leser, M. E.; Miller, R. Lipases at Interfaces: A Review. *Adv. Colloid Interface Sci.* **2009**, *147*−*148*, 237−250.
(46) Sonesson, A. W.; Callisen, T. H.; Brismar, H.; Elofsson, U. M. Adsorption and Activity of Thermomyces lanuginosus Lipase on Hydrophobic and Hydrophilic Surfaces Measured with Dual Polarization Interferometry (DPI) and Confocal Microscopy. *Colloids Surf., B* **2008**, *61* (2), 208−215.
(47) McClements, D. J.; Decker, E. A.; Park, Y. Controlling Lipid Bioavailability through Physicochemical and Structural Approaches. *Crit. Rev. Food Sci. Nutr.* **2008**, *49* (1), 48−67.
(48) Mun, S.; Decker, E. A.; Park, Y.; Weiss, J.; McClements, D. J. Influence of Interfacial Composition on in Vitro Digestibility of Emulsified Lipids: Potential Mechanism for Chitosan's Ability to Inhibit Fat Digestion. *Food Biophys.* **2006**, *1* (1), 21−29.
(49) Eydoux, C.; Spinelli, S.; Davis, T. L.; Walker, J. R.; Seitova, A.; Dhe-Paganon, S.; De Caro, A.; Cambillau, C.; Carrière, F. Structure of Human Pancreatic Lipase-Related Protein 2 with the Lid in an Open Conformation. *Biochemistry* **2008**, *47* (36), 9553−9564.
(50) Winkler, F. K.; D'Arcy, A.; Hunziker, W. Structure of Human Pancreatic Lipase. *Nature* **1990**, *343* (6260), 771−774.

DOI: 10.1021/acsami.5b05068
ACS Appl. Mater. Interfaces 2015, 7, 17460−17470