A NEW APPROACH FOR PREPARING SC-514 LOADED PLGA PARTICLES BY SINGLE EMULSION METHOD

Toluleke Oloruntobi Famuyiwa[1,2,3], Joubin Jebelli[1], Zoey Bowers[1], Austin Bentley[3], Davian Caraballo[2], Paulynice Subtil[1], James Kwasi Kumi Diaka[1], Waseem Asghar [1,2]

[1]Department of Biological Sciences, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431, USA; [2]Department of Computer Engineering & Electrical Engineering and Computer Science, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431, USA; [3]Department of Biological Sciences, Broward College, 3501 Davie Road, Davie, FL 33314, USA

ABSTRACT

Poly (lactic-co-glycolic acid) (PLGA) is a non-toxic or non-injurious subdivision of biodegradable polymer that is not capable of causing immunological rejection. PLGA has been successfully used to encapsulate drugs and deliver drugs in various applications. This high success rate can be linked to the fact that PLGA has been approved by FDA (Food and Drug Administration). SC-514 is a relatively new hydrophobic dug, which has been shown to have anti-cancer effects via inhibition of NF-KB dependent gene expression in cancer cells. SC-514 was encapsulated in PLGA nanoparticles via single-emulsion method. The SC-514 loaded PLGA nanoparticles (diameter=49.4nm) synthesized has the potential to increase the bioavailability of SC-514 drug in prostate cancer treatment. Hence, increasing the therapeutic effect of SC-514 in prostate cancer treatment.

INTRODUCTION

During the last decade, polymers have prevailed as drug transporters or carriers for various applications such as controlled anti-cancer treatments [1] [2]. Drug encapsulation is one of the ways to increase therapeutic effect of anti-cancer drugs [3]. SC-514 is a hydrophobic drug that can heavily benefit from polymer encapsulation, as polymer-enveloped SC-514 drugs are shielded from sudden decomposition and are not predispose to active disintegration during circulation in the body. This protection from degradation leads to longer pharmacologic activity, enhanced anti-cancer efficacy, and reduced systemic side effects of SC-514 during cancer treatments [4]–[6].

Poly (lactic-co-glycolic acid) (PLGA) is a common drug delivery polymer because the encapsulated agent (SC-514 drug in this case) is released over an extended period of time to form lactic and glycolic acids [7]. The time and degree of break down can impact properties such as size, charge, and surface display of ligands. The features obtained will determine the specific body regions targeted for viewing during imaging. PLGA is already used in various treatments of human diseases and injuries. Therefore, clinical adaptation in prostate cancer treatment is encouraging [1].

RESULTS

This study explains in detail the formation and characterization of SC-514 loaded PLGA micro particle and nanoparticles formed by single emulsion, using vitamin E-D-α-Tocopherol polyethylene glycol succinate (vitamin E-TPGS) as the emulsifying agent and ethyl acetate as the solvent (EtOAc). Particle morphology and size were determined utilizing both scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Representative SEM images for microparticles (figure 1 and figure 3) produced with varying emulsifier concentration (0.5% w/v, 0.6% w/v, 0.7% w/v, and 0.8% w/v) indicated

varying size of the SC-514 loaded PLGA nanoparticles. Similarly, representative TEM images for SC-514 loaded PLGA nanoparticles at varying emulsifier concentrations (0.6% w/v, 0.7% w/v, 0.8%w/v) were shown in figure 4.

SC-514 Loaded PLGA Microparticles
Analysis using the dynamic light scattering instrument (Zetasizer Nano-ZS90) produced particles with the following characteristics: Zeta-potential= -54.5mV, Average size (diameter)= 887.9nm, Polydispersity index= 0.195, Count rate=287,370 cps (count per second).The size of SC-514 loaded PLGA microparticles formed were too big for targeted tumor therapy applications. In fact, many studies have shown that for a particle to pass through the blood circulation, the size of the particle must be less than 100nm in diameter [8] [9] [10]. Therefore, we adjusted the parameters used in preparation of SC-514 loaded PLGA microparticles to achieve small-sized microparticles (figure 3) and small-sized nanoparticles (figure 4).

SC-514 Loaded PLGA Nanoparticles
Particle analysis was performed using dynamic light scattering instrument (Zetasizer Nano-ZS90). The analysis of SC-514 loaded PLGA nanoparticles prepared indicated the following characteristics: Zeta-potential= -25.5mV, Average size (diameter) = 49.4nm, Polydispersity index= 0.859, Count Rate = 109,600 cps (count per seconds).

SEM images of SC-514 loaded PLGA microparticles appeared as separate, dissimilar spheres, with unwrinkled surface structure and different sizes throughout the samples (figure 1 and figure 3). Measurements were recorded from the SEM Imager (Quanta, 200). Data from the measurements were utilized to calculate the average diameter of each group. A minimum of 150 diameters were chosen haphazardly to ensure a fair representation of size distribution. The average size of the particles in the group were

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regulated by the combination of fabricated materials utilized. The average diameter of the microparticles in figure 1 was 347.9µm ±370 when 1.5 ml of solvent, 120 mg of PLGA, and 0.4% w/v Vitamin E-TPGS were the fabrication materials utilized. The average diameter of microparticles in figure 3 was 32.5µm ±10 when 1.5 ml of solvent, 120 mg of PLGA, and 0.6% w/v-0.8% w/v Vitamin E-TPGS were the fabrication materials utilized. Interestingly, elevated concentrations of Vitamin E-TPGS, up to 0.7% w/v, produced smaller particles, with average diameters ranging between 200-300 nm (figure 2E). However, increasing the concentration of Vitamin E-TPGS from 0.7% w/v to 0.8% w/v did not decrease the size of PLGA nanoparticles further, rather a sheet like SC-514 loaded PLGA nanoparticles was formed (figure 3).

Higher speeds of centrifugation to 10,000xg (figure 2D) and/or longer centrifugation time of the SC-514 loaded PLGA nanoparticles to 5 hours (figure 2C) improved the yield by increasing the collection of the ultra-small fraction of nanoparticles. Higher speeds and/or longer centrifugation times also allowed the nanoparticles to become less dense, resulting in less particles being lost during washing.

In this study we sonicated the emulsified polymer using an ultrasonicator (amplitude=20, power= 20 to 22 watts) for 30minutes to produce nanoparticles without defect in the morphology and appearance. To further reduce the size of the PLGA nanoparticles, we sonicated for 1 hour and 2 hours at separate times. We observed that the sonication for 1 hour and 2 hours both produced burned or damaged SC-514 loaded PLGA nanoparticles based on the colors of nanoparticles (pink for 30 minutes sonication, light grey for 1hr sonication, and deep grey for 2hrs sonication) produced after sonication (figure 5).

This study also revealed that higher beam strengths may result in the regional heating of the sample, which altered the surface morphology of the particles (figure 1 and figure 3). Additionally, it was noted that microparticles were observable at 100X magnification whereas nanoparticles were distinguishable at 2000X and 3000X magnification.

DISCUSSION

The major challenge encountered in this study was that the size of the SC-514 loaded PLGA particles constructed initially were too big to pass through the circulatory system and would not be useful in cancer Nanomedicine at the clinical stage. Thus, we had to modify our protocol based on suggestions from previous studies [11] [1].

Previous studies indicated that longer centrifugation times resulted in the collection of smaller nanoparticles [1] [12]. This study, therefore, increased the centrifugation time in comparison to protocols performed in other studies [1] [13], which utilized lower centrifugation times. Specifically, this study increased the centrifugation time to 5 hours. We observed that increasing the centrifugation time had the strongest impact on reducing the nanoparticle size as shown in figure 2.

To further reduce the size of the nanoparticles, the sonication time was increased. It was observed that running sonication for a total of 1hour, 30s on and off reduced the size of 3-Bromopyruvate (3-BPA) loaded nanoparticle (unpublished results) but did not reduce the size of SC-514 loaded nanoparticles below the size observed at 30 minutes sonication. 3-Bromopyruvate was more resistant to heat in comparison to SC-514 because of its hydrophilic nature which prevents clumping of the particles. Thus, 3-Bromopuruvate was not burnt after 1hour of sonication while SC-514 was burnt and damaged. This occurred because SC-514 is hydrophobic and could not bond with the water molecules and the increasing collision after 30 minutes favors aggregation of the SC-514 loaded PLGA particles instead of breaking the particles.

This suggests that sonication for 30 minutes on and off is optimum for SC-514 loaded PLGA nanoparticle preparation.

This study examined and modified the variables in previous research protocols which enabled construction of SC-514 loaded PLGA particles. As indicated, the average diameters of these particles range from 49.4nm to 500μm. The techniques for fabricating SC-514 loaded PLGA particles can be altered to obtain the desired particle design, which is specific and compatible with the delivery application of interest. For example, drug delivery in prostate cancer treatment.

As discussed previously, this research specifically modified the parameters used in SC-514 loaded PLGA particles preparation in order to reduce the size of the particles. This study revealed that centrifugation time had the highest impact on PLGA nanoparticle size reduction (**figure 2C**). There was a strong positive correlation between the size of PLGA nanoparticles and zeta potential (**figure 2F**). The size of PLGA nanoparticle decreased as emulsifying concentrations increased from 0.4% w/v to 0.7% w/v. However, increasing the concentration from 0.7% w/v to 0.8% w/v failed to decrease the size of PLGA nanoparticles (**figure 2 E**). Emulsifier concentrations were not increased beyond 0.8% w/v, as a previous study showed the formation of heavy fusing and sheet-like structure was formed when 1% w/v of Vitamin E TPGS was used [1].

In this study, we observed heavy sheet-like structure at 0.8% w/v of emulsifying concentrations (**figure 3**). Partial sheet-like structures were formed when 0.7% w/v of SC-514 loaded PLGA particles vitamin E-TPGS was utilized. Sheet-like structures were not observed when 0.6% w/v vitamin E-TPGS were utilized. This study, therefore recommends 0.6% w/v of vitamin E TPGS for encapsulating hydrophobic drugs such as SC-514 in PLGA nanoparticles.

The particles in this research were produced using TPGS as an emulsifying agent and ethyl acetate (EtOAc) as the solvent. However, it is important to note that there are other solvents and/or emulsifying agents that can be utilized to produce these nanoparticles. In most cases, EtOAc is the solvent of choice as it produces smaller and more evenly sized nanoparticles than dichloromethane (DCM). This effect occurs because EtOAc is completely miscible with water. There are other excellent replacements for EtOAc in some cases [1]. Regardless of the chemical characteristics of the solvent or the emulsifying agent used, an ideal nanoparticle sample must consist of a homogenous glazing of particles around a sphere. However, unusual structures may be formed after freeze drying of the nanoparticles. This study processed at least ten images per group to represent averages of particles size and morphology per trial.

The reliability and consistency of images collected from SEM imaging of PLGA nanoparticles is almost impossible because of the changes in equipment and imaging steps. The differences in the properties of the particles are caused by using different: techniques of emulsification, reagent groups, and equipment.

It is also important to differentiate structural characteristics that are problematic from artifacts that are added unintentionally during imaging. Unfortunately, structural deformations of particles are periodically observed in the SEM images. These structural defects often suggest that the particles were not formed properly. The emulsifier concentration was too low (0.4% w/v) to form discrete particles. At another instance the emulsifier concentration was too high (0.8% w/v) to form discrete particles (**figure 1 and figure 3**). Technical defects are potentially added during the preparation steps and during glazing with gold palladium (**figure 1 and figure 3**).

As mentioned earlier, direct heating from the SEM beam can alter the surface structure of the particles

during SEM imaging. To avoid formation of imaging defects, time spent glazing with gold palladium should be less than 70 seconds, and the strength of the beam power should be less than 60kV. Prolonged glazing times can induce fusing of particles. However, particles with unwrinkled surface structures are formed. Glazing times that are too short could result in difficult imaging because short glazing time leads to unwanted defects on the particle surface during imaging. Extended beam exposure time and deformed images can be prevented by capturing images at short intervals. If the particle surfaces come in contact with the high beam, there will be expansion and cracking of the particles [14]. In other situations, high ultra-sonication power, incomplete evaporation of solvent, incomplete resuspension during the wash phase, or unsuccessful freeze drying may lead to overheating and eventually to fusing of the particles.

The nature of the encapsulant (hydrophobic or hydrophilic) determines the encapsulation method (single- or double-emulsion) of PLGA particles. In this study, the single emulsion permitted particle customization and characterization such that the size, encapsulant, and surface properties were regulated accordingly[7]. This regulation can be achieved by changing the parameters, for example solvent type, emulsification method, and emulsifier type. Absolute mixing of the organic and aqueous phases is required for constructing particle size within the desired range. To achieve complete integration between organic and aqueous phases, some studies utilized either vitamin E-TPGS [15], PVA [16], spans [17], or poloxamers [18]. The images from this study indicated an excellent integration between organic and aqueous phases during preparation of nanoparticles.

The successful preparation of nanoparticles is dependent on the effectiveness of the emulsification process. Polymers that are mixed properly together to form an emulsion will be homogenous and milky-white in appearance. A failed emulsion will consist of extremely large particles of varying size, shape and texture. The mixture usually separates into different layers in the tube [19] [1]. This generally suggests that nanoparticles that are not properly formed, will release the encapsulant. The particles formed may break, if it is not transferred to the ultrasonicator immediately [1]. There could be differences in the physical properties and the cohesive ability of the pellet formed from the particles. Some pellets may break down to suspension after mild vortexing, while others may take several minutes of water bath sonication. The larger the size of the nanoparticles, the higher the volume of SC-514 that will be encapsulated. However, drug release pharmacokinetics may be less consistent in large-sized nanoparticles [20] [21] [22]. The size of nanoparticles often determines the mechanism of particle absorption by cells and their transport around the tissue. The size and distribution in the tissue impact delivery effectiveness and release period.

Subsequent studies will compare the drug delivery efficacy between SC-514 loaded PLGA nanoparticles and SC-514 loaded PLGA micro particles. One of the major factors that determines the efficacy of SC-514 drug delivery at tumor sites is the size of SC-514 loaded PLGA nanoparticles. SC-514 loaded PLGA particles that are larger than 1μM (1000 mM) in the blood circulation will be captured and stagnated by the mononuclear phagocyte system [23] [1]. Small particles that are less than 1μM are capable of immediate infusion across the blood brain barrier or extracellular matrix. On the other hand, large particles would be captured in the blood brain barrier or extracellular matrix. Some studies suggests that delivery of small nanoparticles might support passive targeting of tumors via the enhanced permeation and retention effect [23] [1]. Drug encapsulation efficiency may increase via enhanced permeation and retention.

The efficiency of drug encapsulation is controlled by the properties of the specific drug, the size of the particle, and the chemical properties of the emulsifier. The technique of preparation (single versus double emulsion) impacts drug loading efficiency and nanoparticle size. During encapsulation of hydrophobic agents using single emulsion method rather than double emulsion method , ultra-small nanoparticles were produced [24–29] [8], [30].

The single emulsion method permits changing of formulation variables. Each variable possesses the capability to modify nanoparticle properties. This can be illustrated when DCM is utilized as a solvent instead of ethyl acetate (EtOAc). EtOAc mostly produces larger nanoparticles with a broader size distribution [1]. The force per unit area of the polymer droplet in the main emulsion is diminished, releasing nanoparticles of smaller size because EtOAc is miscible in water [1]. These smaller sized SC-514 loaded PLGA nanoparticles formed indicated that these nanoparticles formed can pass through the vascular or blood circulatory system.

In conclusion, increasing the centrifugation time to 5 hours had the highest impact on SC-514 loaded PLGA particle size reduction. SC-514 loaded PLGA particles potentially possess high drug efficiency and effective drug release pharmacokinetics, if the particle size is appropriate for the application. SEM and TEM studies suggested that the morphology and size of SC-514 loaded PLGA particles formed in this study was appropriate to keep the SC-514 drug potent until the SC-514 drug is released. Thus, PLGA particles loaded with SC-514 drug has the potential to solve the problem of low solubility or low bioavailability of SC-514 drug during prostate cancer treatments.

EXPERIMENTAL PROCEDURES
A technique to enclose hydrophobic drugs such as SC-514 in particle form is single mixing method also known as oil-water technique. In this study, PLGA polymer was transferred into an oil phase (organic) and allowed to dissolve. The resulting mixture was emulsified with a surface-active substance (water) in the aqueous phase. Hydrophobic SC-514 (100µl of 1000µM SC-514 at 25°C) was added directly to the organic phase. Sonication at high power aided the construction of small-sized polymer droplets.

The resulting emulsion was transferred to a larger aqueous phase and stirred for a desired number of hours, which facilitated solvent evaporation. At the end of solvent evaporation, particles were washed and collected via centrifugation. After centrifugation, the particles were freeze dried for long-term storage. The polymer disintegrated gradually via hydrolysis, when SC-514 loaded PLGA nanoparticles were mixed with aqueous environment. The SC-514 drug (encapsulated agent or encapsulant) was released from the nanoparticles over a period of weeks.

A. Preparation of SC-514 Loaded PLGA Nanoparticles
1. 120 mg (+/- 10 mg) of poly (lactic-co-glycolic acid) PLGA was weighed and placed in a 13 mm x 100 mm test tube.

2. 1.5 ml ethyl acetate (EtOAc) solvent was transferred to the sample with a glass serological pipette.

3. The test tube was then covered with a small piece of aluminum foil and subsequently sealed with parafilm to prevent the entrance of air, exposure to air and exposure to light.

4. The polymer was then allowed to dissolve.

5. The work area under the fume hood was prepared with the following equipment/ materials: a vortex mixer, two small pasteurized pipettes with rubber bulbs, and a magnetic stir plate (Thermolyne, Model number SP46615, volts = 120, AMPS = 3.3.,

WATTS = 395, Hz = 60, Phase = 1). The ultrasonicator was in very close proximity to the fume hood. A large beaker, filled with ice, was placed on a stand below the ultrasonicator.

6. 0.6 % w/v Vitamin E-TPGS (50 ml) was added to a 250 ml glass beaker and a magnetic stir bar was placed inside the beaker to facilitate mixing.

7. 0.6% w/v Vitamin E-TPGS (3 ml) was transferred to a glass test tube.

8. 100 µl of SC-514 was added directly to the polymer solution and vortexed until the encapsulant was homogenously dispersed. SC-514 solution was added to the surface of the polymer solution. The sample was ultrasonicated briefly to emulsify the drug with the polymer to create a homogenous, opaque solution. Sonication step was performed on ice.

9. A glass pasteurized pipette was used to add 2ml of polymer solution in a drop wise manner to the vitamin E-TPGS. Polymer/encapsulant mixture was vortexed for 40 seconds until an emulsion was formed.

10. The emulsified polymer was immediately transferred to the ultrasonicator (Misonix Ultrasonic Liquid Processor XL-2000 Series: 20% amplitude for a 20-22 W, 1/8 in probe tip size). The test tube was immersed in ice water and the emulsion was sonicated in 30 seconds bursts for 30 minutes. To ensure even sonication, the test tube containing the emulsion was moved up and down while the probe was inside the emulsion. It was important to avoid contact between the probe and the sides or bottom off the test tube. Moreover, to allow the emulsion solution to cool, there was a pause between every thirty seconds of sonification.

11. 3-4 ml of 0.6 % w/v Vitamin E-TPGS from the stirring solution was transferred to the emulsion using a sterilized pipette (glass material). The mixture formed was less dense and more fluid.

12. The emulsion was poured into the stirring solution.

13. The nanoparticles were stirred continuously for four hours, which facilitated hardening of the nanoparticles. The beaker containing the SC-514 drug was covered with aluminum foil (on the sides and bottom) as SC-514 will diminish in potency if exposed to light.

B. SC-514 Loaded PLGA Nanoparticle Collection

1. The hardened nanoparticles were split into two centrifuge tubes (30 ml nominal volume) and balanced within 0.2 grams on opposite sides of the centrifuge allowing approximately equal weight on opposite sides.

2. The nanoparticles were centrifuged in a fixed-angle rotator for 5hours at 10,000 xg in a micro-centrifuge tube.

3. The supernatant was discarded carefully in a way to prevent any disturbances to the nanoparticle pellets. 10 ml of DI H_2O was added to each pellet in which the centrifuge tubes were placed in a water bath sonicator to completely resuspend the nanoparticles.

4. The contents in both centrifuge tubes were then placed into one centrifuge tube. Steps 2.3 were repeated twice more (rinsed for 15 minutes) for a total of two washes in 20 ml H_2O. The fluid volume of the last pellet resuspension was about 3-4 ml in volume.

Note: In this study, a weight ratio 1:2 trehalose polymer was added as a cryoprotectant to prevent the formation of ice crystals during the freezing process.

5. The nanoparticles were transferred to a pre-weighted 5 ml centrifuge tube and frozen at -80°C for a minimum of 25 minutes.

6. To prevent the frozen contents from melting, the tube was uncapped and covered with paper towel and then secured with a rubber band.

7. The nanoparticle contents were then lyophilized for 72 hours for a 5 ml nanoparticle solution.

8. The centrifuge tube containing the lyophilized particles were then wrapped in parafilm and placed at -80°C for long term storage.

Sizing and Surface Morphology

SEM imaging was done as indicated below:

1. A thin layer of black and grey sided carbon tape was placed on a SEM stub. A marker was utilized to demarcate the metal portion of the stub.

2. A spatula was used to transfer a small quantity of lyophilized SC-514 loaded particles from the micro centrifuge tube unto the surface of the carbon tape. Paper towel was used to clean the surface of the sub to remove the nanoparticles that are not properly attached to the surface of the stub.

Gold-palladium was utilized to glaze the samples for 50 – 150 seconds. This study utilized the following parameters to visualize unwrinkled particles: working distance of 5-20 mm, beam strength of 5-15 kV, and spot size of 1.5.

Dynamic light scattering instrument (Zetasizer Nano-ZS90) was used to investigate the characteristics of the nanoparticles formed. Transmission Electron Microscopy (Philip, CM 200) was performed on the SC-514 loaded PLGA nanoparticles to further investigate the morphology of the nanoparticles in detail.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest with the contents of this article.

REFERENCES

[1] R.L. Mccall, R.W. Sirianni, "PLGA Nanoparticles Formed by Single-or Double-emulsion with Vitamin E- TPGS Video Link," J. Vis. Exp, vol. 82, 2013.

[2] W. Jiang, R.K. Gupta, M.C. Deshpande, S. P. Schwendeman, "Biodegradable poly (lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens," Advanced Drug Delivery Reviews, vol. 57, no. 3 SPEC. ISS. Elsevier B.V., pp. 391–410, 10-Jan-2005.

[3] T.O.B. Olusanya, R. R. H. Ahmad, D. M. Ibegbu, J. R. Smith, A. A. Elkordy, "Liposomal drug delivery systems and anticancer drugs," Molecules, vol. 23, no. 4. MDPI AG, 2018.

[4] J.C. Kaczmarek, "Polymer Lipid Nanoparticles for Systemic Delivery of mRNA

to the Lungs HHS Public Access," Angew Chem Int Ed Engl, 2016.

[5] R. Tong, J. Cheng, "Anticancer polymeric nanomedicines," Polym. Rev., vol. 47, no. 3, pp. 345–381, Jul. 2007.

[6] A. Kumari, S.K. Yadav, S.C. Yadav, "Biodegradable polymeric nanoparticles based drug delivery systems." Colloids Surf. B. Biointerfaces, 2010.

[7] F. Danhier, E. Ansorena, J.M. Silva, R. Coco, A.Le Breton, V. Préat, "PLGA-based nanoparticles: An overview of biomedical applications," Journal of Controlled Release, vol. 161, no. 2. pp. 505–522, 2012.

[8] R. Bhattacharya, T.K. Das, S. Saha, "Synthesis and characterization of CdS nanoparticles," J. Mater. Sci. Mater. Electron. vol. 22, no. 12, pp. 1761–1765, Dec. 2011.

[9] N. Hoshyar, S. Gray, H. Han, G. Bao, "The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction," Nanomedicine. 2016.

[10] G. Fullstone, J. Wood, M. Holcombe, G. Battaglia, "Modelling the transport of nanoparticles under blood flow using an agent-based approach," Sci. Rep., 2015.

[11] S. Tran, P.J. DeGiovanni, B. Piel, P. Rai, "Cancer nanomedicine: a review of recent success in drug delivery," Clin. Transl. Med., 2017.

[12] J.D. Robertson, "Purification of Nanoparticles by Size and Shape," Sci. Rep., 2016.

[13] M.S. Cartiera, E.C. Ferreira, C. Caputo, M.E. Egan, M.J. Caplan, W.M. Saltzman, "Partial correction of cystic fibrosis defects with PLGA nanoparticles encapsulating curcumin," Mol. Pharm., 2010.

[14] J.A. Meganck, K.M. Kozloff, M.M. Thornton, S.M. Broski, S.A. Goldstein, "Beam hardening artifacts in micro-computed tomography scanning can be reduced by X-ray beam filtration and the resulting images can be used to accurately measure BMD," Bone, 2009.

[15] L. Mu, S.S. Feng, "A novel controlled release formulation for the anticancer drug paclitaxel (Taxol®): PLGA nanoparticles containing vitamin E TPGS," J. Control. Release, 2003.

[16] M.S. Cartiera, K.M. Johnson, V. Rajendran, M.J. Caplan, W.M. Saltzman, "The uptake and intracellular fate of PLGA nanoparticles in epithelial cells," Biomaterials, 2009.

[17] T. Niwa, H. Takeuchi, T. Hino, M. Nohara, Y. Kawashima, "Biodegradable submicron carriers for peptide drugs: Preparation of dl-lactide/glycolide copolymer (PLGA) nanospheres with nafarelin acetate by a novel emulsion-phase separation method in an oil system," Int. J. Pharm., 1995.

[18] T. Ameller, V. Marsaud, P. Legrand, R. Gref, G. Barratt, J.M. Renoir, "Polyester-poly(ethylene glycol) nanoparticles loaded with the pure antiestrogen RU 58668: Physicochemical and opsonization properties," Pharm. Res., 2003.

[19] C.I.C. Crucho, M.T. Barros, "Polymeric nanoparticles: A study on the preparation variables and characterization methods," Materials Science and Engineering C. 2017.

[20] R.P. Batycky, J. Hanes, R. Langer, D.A. Edwards, "A theoretical model of erosion and macromolecular drug release from biodegrading microspheres," J. Pharm. Sci., 1997.

[21] J. Panyam, "Polymer degradation and in vitro release of a model protein from poly (D,L-

lactide-co-glycolide) nano- and micro particles," J. Control. Release, 2003.

[22] J. Siepmann, N. Faisant, J. Akiki, J. Richard, J.P. Benoit, "Effect of the size of biodegradable microparticles on drug release: Experiment and theory," J. Control. Release, 2004.

[23] N. Bertrand, J. Wu, X. Xu, N. Kamaly, O.C. Farokhzad, "Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology," Advanced Drug Delivery Reviews. 2014.

[24] J.M. Barichello, M. Morishita, K. Takayama, T. Nagai, "Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by the nanoprecipitation method," Drug Dev. Ind. Pharm., 1999.

[25] R.C. Mundargi, V.R. Babu, V. Rangaswamy, P. Patel, T.M. Aminabhavi, "Nano/micro technologies for delivering macromolecular therapeutics using poly(d,l-lactide-co-glycolide) and its derivatives," Journal of Controlled Release. 2008.

[26] T. Niwa, H. Takeuchi, T. Hino, N. Kunou, Y. Kawashima, "Preparations of biodegradable nanospheres of water-soluble and insoluble drugs with D, L-lactide/ glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior," J. Control. Release, 1993.

[27] Y.Y. Yang, H.H. Chia, T.S. Chung, "Effect of preparation temperature on the characteristics and release profiles of PLGA microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method," J. Control. Release, 2000.

[28] Y.Y. Yang, T.S. Chung, N. Ping Ng, "Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method," Biomaterials, 2001.

[29] Z. Zhang, S.S. Feng, "The drug encapsulation efficiency, in vitro drug release, cellular uptake and cytotoxicity of paclitaxel-loaded poly (lactide)-tocopheryl polyethylene glycol succinate nanoparticles," Biomaterials, 2006.

[30] R.L. McCall, R.W. Sirianni, "PLGA Nanoparticles Formed by Single- or Double-emulsion with Vitamin E-TPGS," J. Vis. Exp., 2013.

[31] E.M. Collnot, "Mechanism of inhibition of P-glycoprotein mediated efflux by vitamin E TPGS: Influence on ATPase activity and membrane fluidity," Mol. Pharm., 2007.

EXPERIMENTAL FIGURES
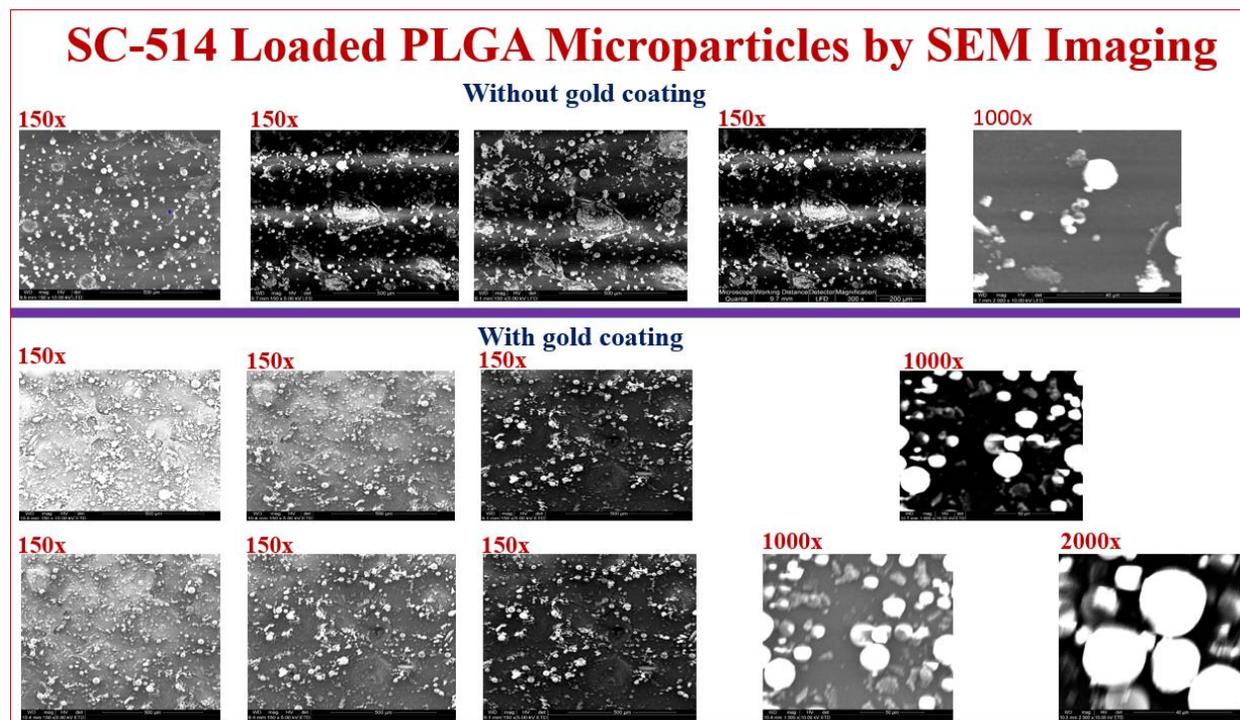### Scanning Electron Microscopy (SEM)

Figure 1: Non coated SC-514 loaded PLGA micro particle were not distinct in appearance as depicted in the images shown at 150x and 1000x magnification. Interestingly, the background was intact and clear because there was no heat released as a result of coating with gold palladium. Conversely, gold-coated SC-514 loaded PLGA micro particle were distinct in appearance as shown in the 150x, 1000x and 2000x magnification images. However, the background was not clear as the heat released from the gold coating melted the background to some extent.

Varying SC-514 Loaded PLGA Micro particles' Parameters to Achieve Smaller-Sized Particles
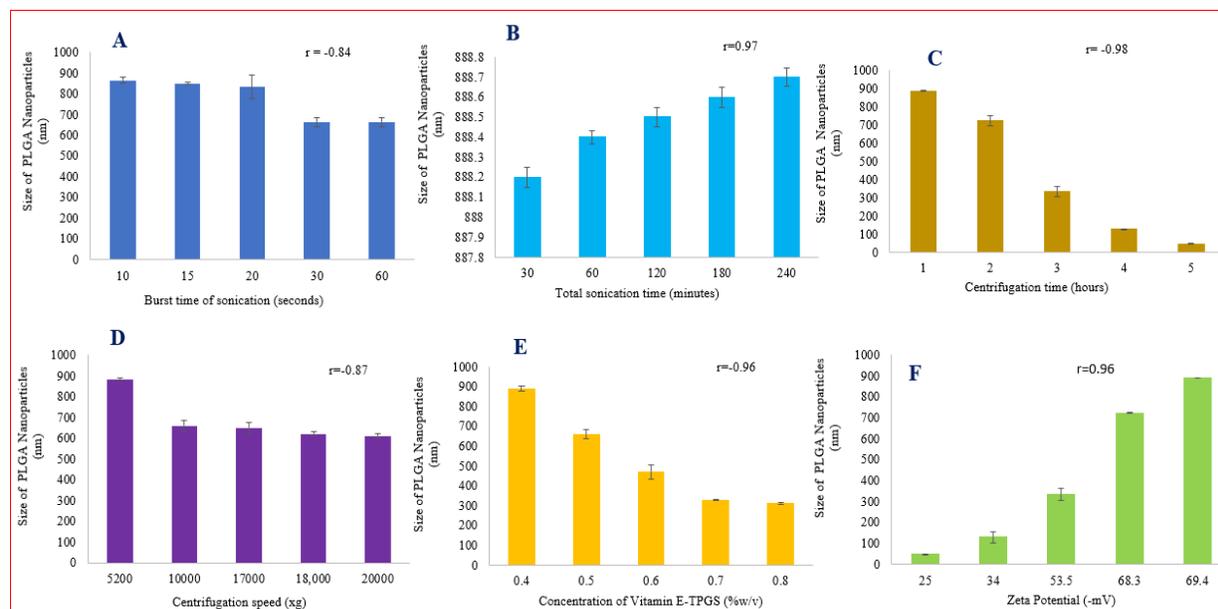
Figure 2: Varying SC-514 loaded PLGA microparticles parameters to achieve smaller-sized nanoparticles. **A**: The size of PLGA nanoparticle decreased when burst sonication increased from 20 to 30 seconds. The size of PLGA nanoparticle did not decrease at other burst time changes. **B:** The size of PLGA nanoparticles increased consistently as the total sonication time increased. **C:** Centrifugation time appeared to have the highest impact on PLGA nanoparticle size reduction. There was a strong negative correlation between PLGA nanoparticles size and centrifugation time. **D:** The size of SC-514 loaded PLGA nanoparticles decreased as the Centrifugation speed increased from 5200xg to 10000xg. However, the size of PLGA nanoparticle did not change when centrifugation speed increases from 10000xg to 20000xg. **E:** The size of PLGA nanoparticle decreased as emulsifying concentrations increased from 0.4% w/v to 0.7% w/v. interestingly, increasing the concentration from 0.7% w/v to 0.8% w/v did not decrease the size of PLGA nanoparticles. **F:** There was a strong positive correlation between the size of PLGA nanoparticles and magnitude of the zeta potential.

After varying the parameters used for the preparation of SC-514 loaded PLGA micro particle, we were able to successfully construct a smaller-sized SC-514 loaded PLGA microparticles (**figure 3**) and smaller-sized SC-514 loaded PLGA nanoparticles (**figure 4**). The characteristics of these new particles were different. In accordance with our naming system, most studies described these new particles as nanoparticles (particle size less than 100nm) while the former particles were described as microparticles (particle size greater than 100nm) based on the difference in the sizes of the particles [1], [10], [11].
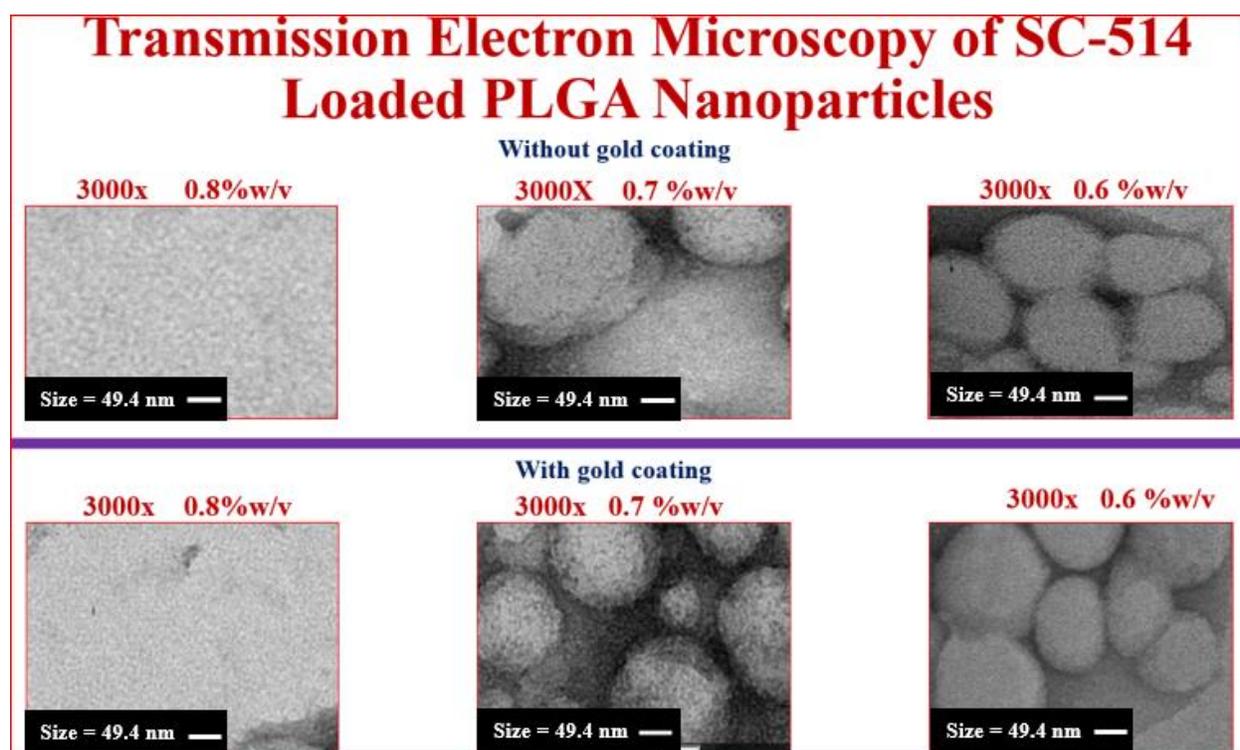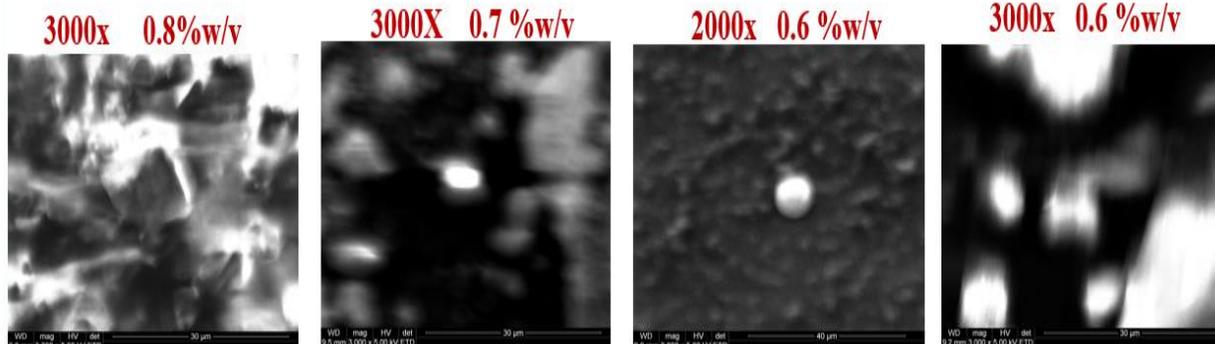

Figure 3: Gold coating SC-514 loaded PLGA microparticles enhanced the characteristics of SC-514 loaded PLGA micro particles. Gold-coating with gold palladium made SC-514 loaded PLGA microparticles more distinct in comparison to the background. SC-514 loaded PLGA microparticles appeared most distinct at 0.6% w/v of the emulsifying concentration. SC-514 loaded PLGA microparticles were observable at 0.7% w/v of the emulsifying concentration. Interestingly, emulsifier concentrations greater than 0.7% w/v Vitamin E-TPGS completely changed the morphology of both the gold-coated and non-coated microparticles to sheet-like structures.
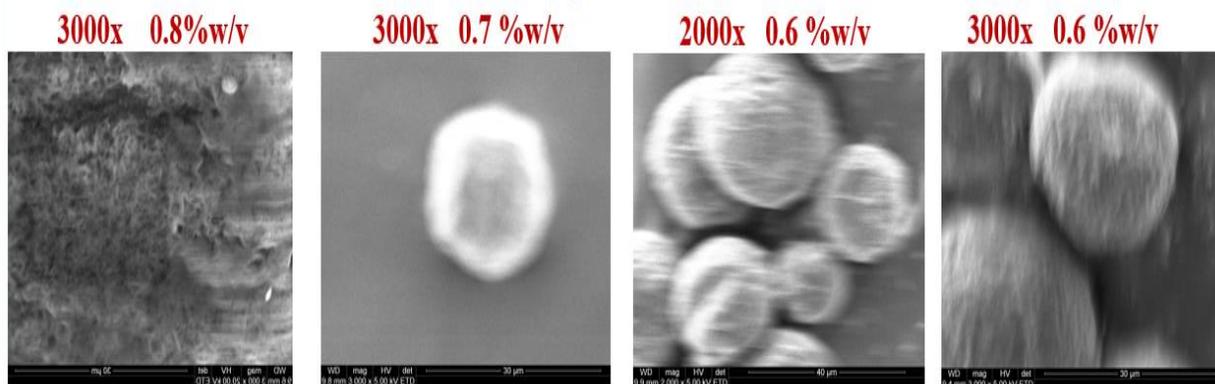
Figure 4: Images from Transmission Electron Microscopy suggested that gold-coating enhanced the characteristics of SC-514 loaded PLGA nanoparticles. Gold-coating with gold palladium made SC-514 loaded PLGA nanoparticles to be more distinguishable in comparison to the background. SC-514 loaded PLGA nanoparticles appeared most observable at 0.6% w/v of the emulsifying concentration. SC-514 loaded PLGA nanoparticles was observable at 0.7% w/v of the emulsifying concentration but was not as clear as the images at 0.6% w/v of the emulsifying concentration. Emulsifier concentration at 0.8% w/v Vitamin E-TPGS completely changed the morphology of the nanoparticles to a sheet-like structure.
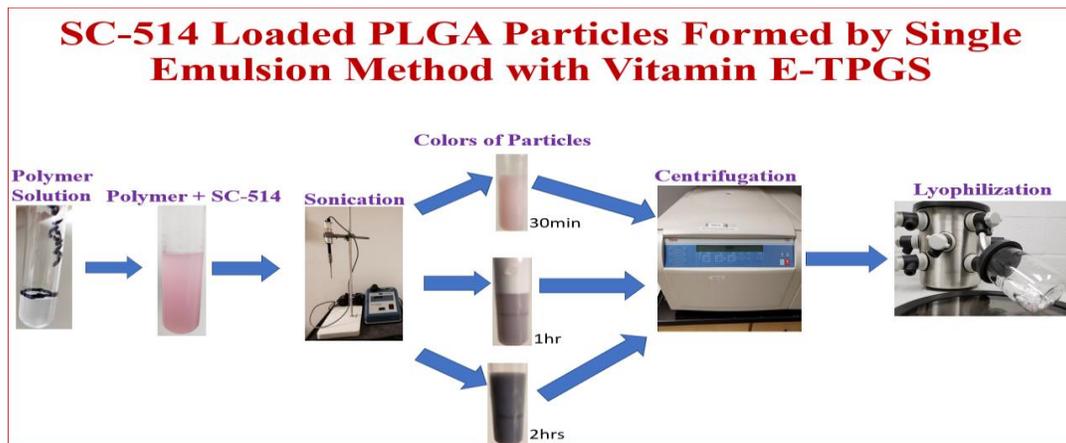
Flow Chart of Materials and Method

Figure 5: SC-514 loaded PLGA particles was formed by single emulsion method with Vitamin E-TPGS: PLGA polymer was dissolved in ethyl acetate to form polymer solution. 100µl SC-514 (1000µM) of drug solution was added into the polymer solution; the formed mixture was then emulsified with Vitamin E-TPGS; the emulsified polymer was then sonicated using the ultrasonicator for 30minutes. The particles were allowed to harden as the solvent evaporated; the collected particles were then washed and centrifugation at 10,000 rpm for 5hours. SC-514 loaded PLGA nanoparticles formed were then lyophilized for long term storage.
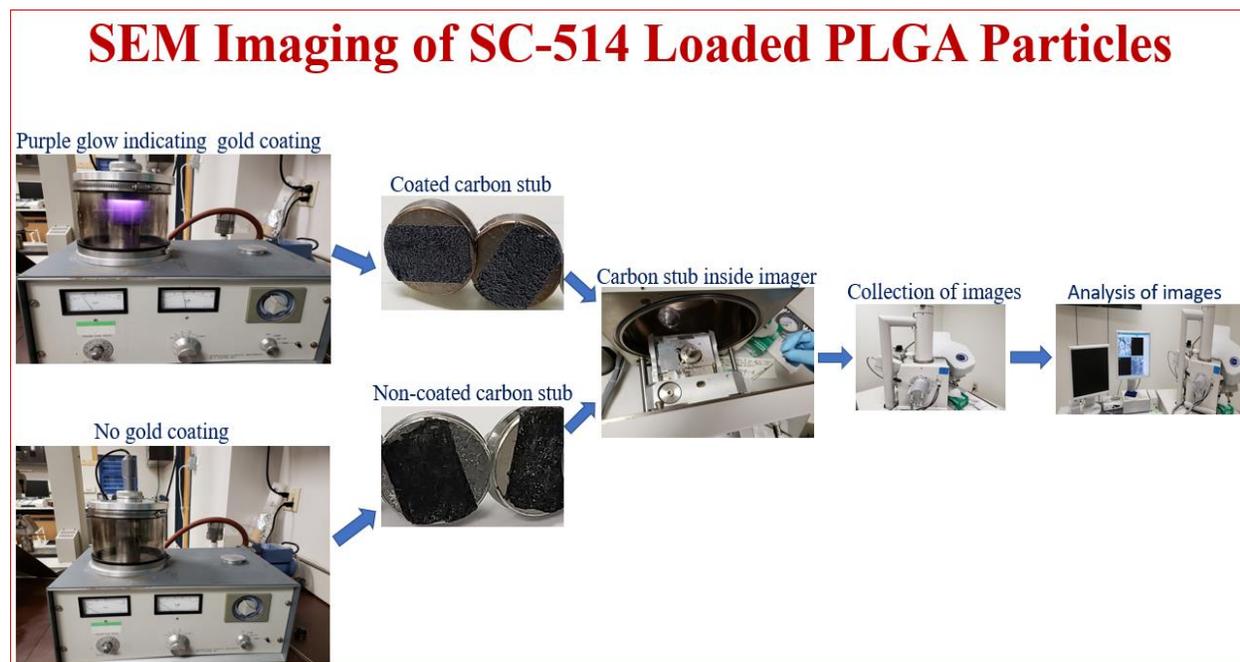

Figure 6: Schematic illustration of scanning electron microscopy (SEM) imaging of SC-514 loaded PLGA nanoparticles. The lyophilized nanoparticles were mounted on a carbon stub. Gold palladium was then used to coat the surface of the nanoparticles in order to enhance the structural characteristics of the SC-514 loaded PLGA nanoparticles. The control group, consisting non-coated nanoparticles were also utilized to compare the data analysis with the gold coated SC-514 loaded PLGA nanoparticles. The carbon stub was inserted into the imager to take picture of the nanoparticles. The pictures were collected, organized and analyzed.