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Original Article

Dexamethasone-Loaded PLGA Microspheres: Effective and Sustained Anticancer Drug Delivery System

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ABSTRACT

Objective: Encapsulation of dexamethasone (DEX) anticancer drug with Poly (d, l-lactide-co-glycolide) (PLGA) microsphere (MS) was carried out for sustained release system. **Methods:** The characterizations of MS were analyzed using XRD, FTIR, RP-HPLC, TEM, CLSM, and XRD patterns and reported the physical state of the encapsulated PLGA microsphere (DEX-PLGA MS). **Results:** The presence of 2 θ peaks values at 33.17°, 35.63°, 49.46° and 54.09° confirmed the semi-crystalline phase for the DEX drug. FTIR showed peaks at 1695 cm^{-1}, 1644 cm^{-1} and 1602 cm^{-1} were shown to be confirming DEX encapsulation. The MTT assay showed a greater cellular uptake of DEX-PLGA MS and the new formulation showed steady and sustained release of DEX resulting in a lower short term cytotoxic effect against HEP-G2 cells. **Conclusion:** In the present study, the drug encapsulation microsphere is non-toxic, simple, and an ideal method for anticancer drug release. The present results revealed that the use of DEX-PLGA MS offers large-scale production of biocompatible microspheres that can be used against HEP-G2 cells.

Key words: Dexamethasone, PLGA, Sustained release, MTT assay, TEM, Confocal laser scanning microscopy.

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INTRODUCTION

Nanotechnology can be defined as the science and technology involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization at the nanometer scale.[1] Nanomedicine involves utilization of nanotechnology for the welfare of human health. In recent years, significant efforts have been made to use nanotechnology in drug and vaccine delivery.[2] This increasing interest in nanomedicine is driven in by the fast pace of innovation and emerging successes of nanoparticle based drug delivery systems. Nanoparticulate polymeric delivery systems have been investigated as a possible approach to increase the oral drug availability.

Poly (d,l-lactide-co-glycolide) (PLGA) is a copolymer which is approved by the Food and Drug Administration (FDA) therapeutic devices, owing to its biodegradability and biocompatibility. PLGA based nanotechnology has gained tremendous interest in medical applications such as sustained drug release, drug delivery, diagnostics and treatment.[3] It protects the encapsulated drug from enzymatic degradation and changes the pharmacokinetics of the drug. It offers a wide range of degradation rates, from months to years, depending upon its composition and molecular weight.[4] PLGA-based nanoparticles are currently under investigations for their applications in cancer imaging and cancer therapy. Biodegradable particulate carrier systems are interest as potential means for oral delivery to enhance drug absorption, improve bioavailability and target therapeutic agents to organ. The drug release can be controlled by the molecular weight of PLGA and the polymerization ratio of lactide to glycolide. Moreover, PLGA has proven to be safe because it decomposes to lactic acid and glycolic acid in the body and is finally excreted as CO_2.[5] Particles composed of PLGA have been employed in various fields of biosciences, such as biomedicine, bioscience, biomaterial, and drug delivery systems.[6] Some findings have suggested an enzymatic role in PLGA breakdown based on the difference in the *in vitro* and *in vivo* degradation rates.[7] Drug release from PLGA microspheres can range from days to months and therefore, accelerated *in vitro* drug release testing methods are often used for manufacturing batch release. However, these methods often involve change in the mechanism of drug release from erosion/diffusion control use of organic solvents which solubilizes the polymer, and lead to diffusion controlled kinetics. This alteration in the mechanism of release may result in a lack of correlation between accelerated release profiles and *in vivo* release profiles. In particular, where multiple release phases occur, and the different phases are frequently lost in accelerated release testing.[8]

Dexamethasone (DEX) a synthetic corticosteroid has been widely used for diagnosis and therapy of various diseases or anomalies in the field of clinical medicine.[9] It is a glucocorticoid that is used clinically as an anti-inflammatory and immunosuppressive agent. Dexamethasone is also used as a direct chemotherapeutic agent in certain hematological malignancies, especially in the treatment of multiple myeloma, in which it is given alone or in combination with other chemotherapeutic drugs, including thalidomide, lenalidomide and bortezomib.[10] The intra-arterial administration of dexamethasone containing nanoparticles has been demonstrated to be effective in reducing restenosis in rat model.[11] Preparation of PLGA nanoparticles loaded with dexamethasone by an exclusive oil-in-water (O/W) emulsion/solvent evaporation method was described by several researchers.[12] The encapsulation of dexamethasone acetate containing magnetic poly (d,l-lactic-co-glycolic acid) (PLGA) nanoparticles were found to be suitable for intra articular injection for the treatment of arthritis or osteoarthritis.[13] Fan *et al.*[14] investigated the incorporation of dexamethasone /poly (d,l-lactic acid) microspheres into poly (ethylene glycol)-poly(ε-caprolactone)-poly (ethylene glycol) hydrogel to prepare an injectable hydrogel composite which has great applications in the orthopedic tissue engineering field. Dexamethasone acetate loaded PLGA particles exhibited higher loading efficiency and linear release profile of drug and sustained release of dexamethasone from hydrophilic matrices using PLGA nanoparticles for neural drug delivery.[15] In the present study, we suggest the encapsulation of DEX

loaded PLGA MS would be useful in aiming at the development of cytotoxicity against HEP-G2 cells. It is an eco-friendly approach for the DEX encapsulated PLGA microspheres.

MATERIALS AND METHODS

MATERIALS

Poly (d,l-lactide-co-glycolide) (PLGA), (50:50 lactic acid: glycolic acid) with an inherent viscosity of 0.63 dl/g (Hexafluoroisopropanol) were purchased from Sigma Aldrich, India. Dexamethasone (DEX) was purchased from Sigma, India, Phosphate buffered saline (pH 7.2, PBS), Poly vinyl alcohol (PVA), Poly ethylene glycol (PEG), Dichloromethane (DCM) and Matrigel (Column) were obtained from Sigma chemical Co Maharashtra, India and MPM Scientific Co., Vellore, India. The chemicals and reagents for HPLC analysis were obtained from Fisher Scientific Co., (Fair Lawn, NJ). Saponin (Internal standard) and DEX were obtained for HPLC assays analysis. All chemicals were used as received.

Study design

Preparation of dexamethasone encapsulated PLGA microspheres

The microspheres (MS) were prepared using oil in water (o/w) emulsion-solvent evaporation technique. Different weights of PLGA were dissolved in 1.5mL of DCM in a screw capped test tube to make the solutions of 7.5-15% (w/v). Weighed amount of DEX was dispersed in the organic phase using sonicated water bath for 10 min. The organic phase was added drop by drop using a Pasteur pipette with 50 mL of an aqueous PVA solution (1, 3, or 5%w/v) in a beaker and stirred at 2000 rpm using an overhead stirrer. The emulsification process was continued for 10 min and the emulsion was stirred at 500 rpm using magnetic stirrer for 3 h to evaporate the organic solvent. Microspheres were obtained by centrifugation at 8000 rpm. The solution was washed 4 times with distilled water, and freeze-dried overnight. The dried microspheres were kept in a desiccated bag and stored at -20°C. Microspheres were prepared using the same procedure except that the additive PEG was dissolved in the organic phase prior to the addition of the drug.[16]

X-ray diffraction (XRD)

XRD measurements were made for studying the crystallinity of DEX encapsulated PLGA microspheres (DEX-PLGA MS) were conducted in an X-ray diffractometer (Philips PW1820) operating with Cu Kaα and Kaα radiations. The scans were recorded in the 2h range of 10°and 80°.

Fourier transforms infrared spectroscopy (FTIR)

FTIR spectroscopy was carried out using KBr disk method. 1-2 mg of sample (DEX encapsulated PLGA microspheres) was mixed with potassium bromide, compressed at a pressure of 6 ton/cm^{-2} into discs and scanned (Thermo Nicolet, Avatar 370 FTIR spectrophotometer) over the range of 4000-400cm^{-1}. Blank KBr pellet was used as a reference.

Determination of dexamethasone by Reverse phase high-performance liquid chromatography (RP-HPLC)

Reverse phase high-performance liquid chromatography (RP-HPLC) is based on the full capabilities of thin layer chromatography. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, and hyphenation. DEX was routinely estimated by a modified HPLC technique. PLGA MS containing drug or standard solutions (calibration curve) were initially frozen dried on a Lab Con Co freeze dryer and 1 mL of the mobile phase (MeOH 6%; tetrahydrofuran 50%; H_2O 44%) were used for sample reconstitution 0.5 mL of the supernatants was mixed with 0.05 mL of 1000 ppm solution of DEX used as internal standard, and 20 μL of each sample was injected in the HPLC column.[17]

Drug release kinetics

10 mg of DEX-PLGA MS were suspended in 6 mL of PBS in screw-capped test tubes. The tubes were kept under constant shaking (60 rpm) in a shaking water bath (Remi, India) at 37°C. The drug release kinetics experiments were carried out under sink condition, where the drug concentration in the release medium was not exceeding 10 % of the saturation concentration. It is important to mention that the saturation solubility of the drug in the buffer used at 37°C was measured in the lab and found to be 0.38 mg/mL, and the concentration of the drug in the release medium at different time intervals of the whole experiment was not exceed 0.03mg/mL. Different time intervals, the tubes were centrifuged, and 5mL were withdrawn from each tube and replaced with 5mL of fresh buffer solution (kept at the same temperature). The drug concentration was determined spectrophotometrically at 780 nm of the withdrawn samples. Release experiments were conducted in triplicate for each batch.[18]

Swelling ratio of DEX- PLGA MS

The swelling ratio of blank MS was determined by immersion method in a phosphate buffered saline (PBS pH 7.4) at room temperature for 48 h with gentle shaking. At specific time points (0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h) samples were removed and rinsed with Milli Q water. The MS remained intact during the process and no macroscopic pores were visible and the shape of MS remained the same. The MS were blotted dry and the swollen weight (W_{SW}) was measured and the swelling ratio (E_{SW}) was calculated according to equation as follows:

$$E_{SW} = \frac{W_{SW} - W_0}{W_0} \times 100$$

Where E_{SW} is the swelling ratio of the MS W_0 is initial dry weight of MS and W_{SW} is the weight of the swollen MS. Swelling ratio was determined using duplicate samples of MS.[19]

Encapsulation efficiency

Weighed MS were dispersed in a small volume of dichloromethane (DCM) and dissolved the polymer. 50 mL of phosphate buffer saline (pH 7.4) was stirred using magnetic stirrer to evaporate DCM. The remaining aqueous medium was centrifuged, and aliquots of the supernatant were analyzed for the drug using UV/Vis spectrophotometer (Varian, Cary 5000) at 780 nm. Drug-free MS were prepared and subjected to the same procedure, and the supernatant obtained was used as a blank. The polymer was found not to interfere with the assay, as the maximum absorbance wavelength, and absorptions were found to be unaffected by these additives.[18] Experiments were carried out in triplicates and the average values reported. Drug encapsulation efficiency percent (EE %) was calculated using the following formula:

$$\text{Encapsulation efficiency \%} = \frac{\text{Actual amount of drug in microspheres}}{\text{Theoretical amount of drugs in microspheres}} \times 100$$

Brine shrimp (BST) assay

The eggs of *Artemia salina* (Leach) were placed in sea water and hatched within 48 h and the larvae were used for the experiments. Brine shrimp larvae (*nauplii*) were tested for DEX-PLGA MS at the concentrations

of 10, 20, 30, 40 and 50 μg/mL in test tubes containing 5 mL of larvae with 10 shrimps in each of three replicates were tested against the brine shrimp larvae. Mortality of larvae were counted after 24 h and the data were processed in a simple program for probit analysis to estimate LC_{50} values with 95% confidence intervals for statistically significant comparisons of potencies.[20]

Cytotoxic effect of DEX-PLGA MS using MTT assay

The cytotoxicity of the DEX-PLGA MS was evaluated using MTT assay. To measure the inhibitory effects of various DEX formulations against cancer cells, the Hep-G2 cells were seeded into 96-well culture plates at 5000 cells/well and cultured at 37°C in a humidified atmosphere with 5% CO_2 for 24 h. The cells were exposed at different concentrations (0-500 μg/mL) of DEX-PLGA MS. The blank culture medium was used as a control without microparticles. The MS were sterilized using UV irradiation for 60 min before use. The cultured cells were assayed for cell viability with MTT after 48 h. The wells were washed twice with PBS and 30 μL of MTT supplemented was added to the culture medium after the unreacted dye was removed 3.5-4 h incubation by aspiration, and 200 μL of DMSO was added to each well to dissolve the dark blue crystal. The absorbance intensity was measured by the microplate reader (Genios, Tecan, Mannedorf, Switzerland) at 570 nm with the background at 660 nm. Cell viability was calculated by the following equation.[21]

$$\text{Cell viability (\%)} = \frac{\text{Int}_s}{\text{Int}_{control}} \times 100\%$$

Where Int_s is the absorbance intensity of the cells incubated with the MS suspension and $Int_{control}$ is the absorbance intensity of the cells incubated with the culture medium only (positive control). The error bars were obtained from triplicate samples.

Transmission electron microscopy (TEM)

The processing of TEM image generally relies on apriori- information about the formation of the image. The prepared sample were analyzed by TEM with carbon-coated TEM grids and the measurements were performed in a JEOL model 1200 EX instrument operated at an accelerating voltage of 120 kV and later with and XDL 3000 powder.

Confocal laser scanning microscopic (CLSM) study

Confocal laser scanning microscopy (CLSM) is a classical technique to generate images from cell or tissue samples by means of laser scanning on an optical platform. The images were obtained at a higher resolution with depth selectivity compared to conventional optical microscopy or fluorescence microscopy.

Rhodamine B chloride was added to organic solution prior to nanoparticles preparation and the MS were labelled adding 1% (w/w) of fluorescent rhodamine B chloride with D6 dimethyl sulphonic acid (D6 DMSO) solution before the spray dried. Glass slides were examined in CLSM, (Zeiss LSM 510 Meta equipped) with 1mW helium neon and an argon laser, using Plane Apo chromat 63× objective (NA1.40, oil immersion). Red fluorescence was observed with 560 nm emission filter under 543 nm laser illuminations. Green fluorescence was observed with 505-550 nm emission filters under 488 nm illuminations and the pinhole diameter was at 104 µm. Stacks of images were collected every 0.8 µm along the Z axis.[22]

RESULTS

X-ray diffraction (XRD)

In the present study, XRD patterns were established to study the physical state of the pure DEX (drug), PLGA (vehicle), and the DEX encapsulated PLGA microsphere (DEX-PLGA MS). The presence of 2 θ peaks values at 33.17°, 35.63°, 49.46° and 54.09° confirmed the semi-crystalline phase for the DEX drug as shown in Figure 1 A, B and C. XRD analysis was used to determine the crystalline content of DEX in the PLGA MS. The FTIR spectra of the DEX-PLGA MS and DEX drug control are shown in Figure 2 A and B. In the present observations revealed that the functional groups peak at 3545 cm^{-1} (hydroxyl O-H stretching), 3419 and 3328 cm^{-1} (-NH stretching vibration), 2942, 2925 and 2851 cm^{-1} (-CH stretching vibrations), 1695 cm^{-1} (-CO stretching vibration of a carboxyl group), 1644 cm^{-1} (-CO stretching vibration of -$CONH_2$ group) and 1602 cm^{-1} (-NH vibration) to be in excited state, when exposed to infrared wavelengths (Figure 2A). 1695 cm^{-1} (-CO stretching vibration of a carboxyl group), 1644 cm^{-1} (-CO stretching vibration of -$CONH_2$ group) and 1602 cm^{-1} (-NH vibration) were shown to be similar to DEX peaks (Figure 2B).

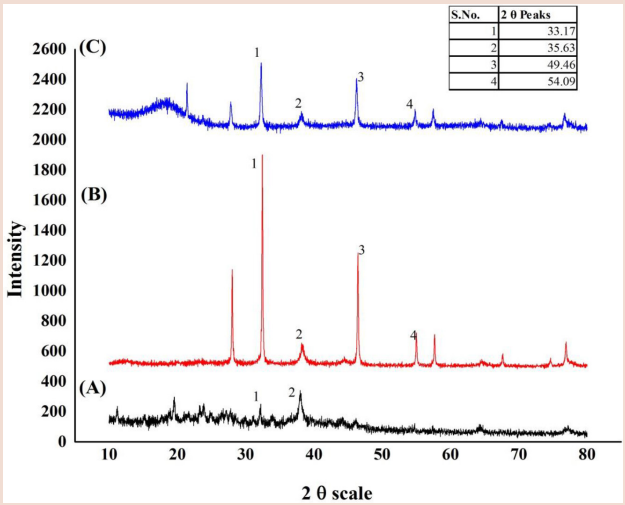

Figure 1: XRD spectrum of (A) PLGA (vehicle) (B) DEX (drug) (C) DEX-PLGA MS.

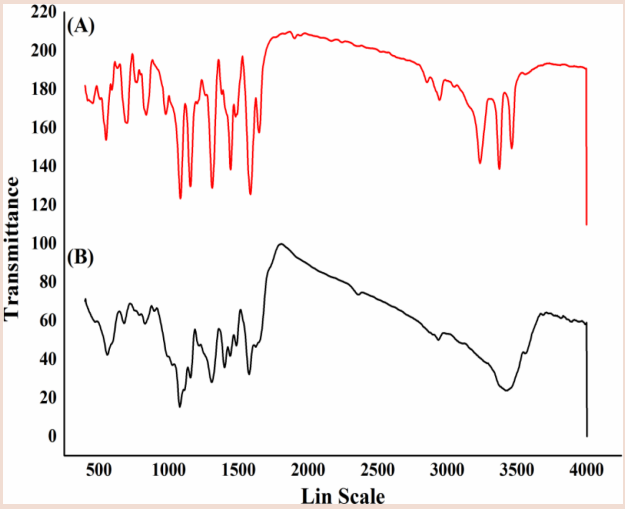

Figure 2: FTIR spectrum of (A) DEX-PLGA MS (B) Dexamethasone drug (control).

Reverse phase-high pressure liquid chromatography (RP-HPLC)

The RP-HPLC method different mobile phases were tried and the phase containing acetonitrile and phosphate buffer (60:40, v/v) were found to be optimal for obtaining well defined and resolved peaks with mean retention time of 3.7 min eluted the DEX from the microsphere. The resultant was found to be corresponds retention time of DEX drug. The retention time for DEX was confirmed for with control steroid (saponin), which affirmed the presence of DEX in the DEX-PLGA MS. The recovery efficiency factor on the calculated encapsulation efficiency was determined as the ratio of the paclitaxel concentration obtained from HPLC to the theoretical concentration of the prepared solution which was obtained by dissolving the physical mixture of pure paclitaxel and placebo nanoparticles with relevant ratio in acetonitrile. In the present study, the resolved peaks with mean retention time of 3.7 min eluted the DEX from the microsphere.

In vitro drug release kinetics

The *in vitro* drug release of DEX-PLGA MS showed three release profiles with an initial burst release, followed by lag phase and then a secondary zero-order phase. Because of the hydrophobic characteristics of DEX in a single emulsion formulation, that the encapsulated substance may be agglomerated toward the MS surface during solvent evaporation. The MS prepared by emulsification solvent evaporation was present an initial burst release due to surface located encapsulated substance. The calculated encapsulation efficiency of DEX (21-31%) was higher compared to control sample. However, the calculated loading of DEX was only 30 μg/mg (drug/polymer) compared to 100μg/mg for control and DEX. The *in vitro* drug release kinetics of the DEX-PLGA MS was calculated for the time of 200 min (Figure 3). The drug release kinetics showed triphasic phase with log phase at 15 min, lag phase at 29th min and stationary phase from 40th to 200th min, leading to a prolonged and sustained phase of drug delivery. The DEX solubility in water was ~5 mg/ml, and the calculated encapsulation efficiency was from 14% to 21%, depended up on the particle size. DEX drug revealed that the different degrees of water solubility were noticed in the encapsulation of PLGA MS. The control peaks were much lower than compared to DEX-PLGA MS, which was due to absence of DEX.

Brine shrimp assay (BST)

The *in vitro* brine shrimp assay is an inexpensive bench top assay mainly used to evaluate the potency of various kinds of drug and nanoformulation. Initially, the DEX-PLGA MS was tested at 1 µg/ml and nil mortality was observed in shrimp larvae after 24 h. The assay was repeated at 2.5, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 µg/mL and the dead shrimp larvae were counted regularly at 1, 2, 3, 4, 20 and 24 h of study for DEX-PLGA MS. The mortality of *nauplii* larvae were counted at 40 µg/mL and showed decrease trend of mobility observed after 24 h incubation with survival frequency of 80 % per test tube. No lethal effect was noticed for brine shrimp at the concentration tested against cancer cells for the encapsulated MS. The survival frequency of *nauplii* larvae tested at various concentrations of DEX-PLGA MS is shown in Figure 4. Towards the end of incubation, the shrimp larvae in control vials were showed in active state, while the others in treated vials appeared very weak in terms of their activity.

Cell proliferation study (MTT assay) of DEX-PLGA MS

To assess the cellular cytotoxicity of the HepG2 cell lines were used. Cytotoxic activity was evaluated at two-fold dilutions in triplicate ranging from 10μg/mL to 100μg/mL. Cell viability was assessed for DEX-PLGA MS by MTT assay after 24 h exposure period. Experimental IC_{50} values of the DEX-PLGA MS tested against HepG2 cells were found to be 68.5 mM (Figure 5). The bioassay for control drug of DEX is shown in Figure 7A. The IC_{50} 3.429 mM for HepG2 cells Figure 6B, C, D and E which translates to ~200-300 mg/kg body weight in an adult human, suggesting low *in vitro* cytotoxicity as it represents a much higher intravenous material dose than required for *in vivo* drug delivery.

DEX-PLGA MS showed the growth inhibition with IC_{50} value of 3.429 mM further reduced by the addition of 50mM DEX. The plaque formation on Hep-G2 cells grown in media with 2% FBS (fetal bovine serum) with increasing order with the concentration order. DEX release from the MS happened either by diffusion through the polymer barriers or by erosion of the polymer materials due to the hydrolytic degradation of ester linkages. Because of the hydrophobic characteristics of DEX in a single emulsion formulation, it was likely that the encapsulated DEX may be agglomerated the MS surface during solvent evaporation. The

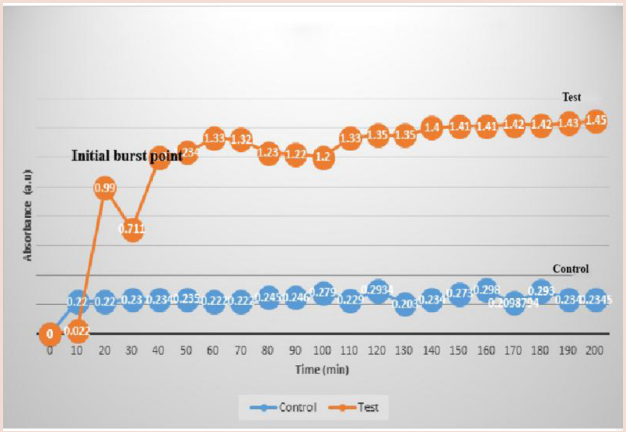

Figure 3: *In vitro* drug release kinetics of DEX-PLGA MS compared with MS (control).

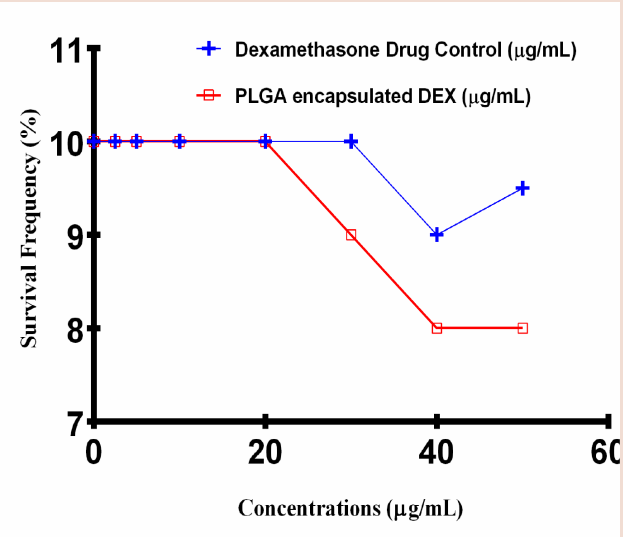

Figure 4: Survival frequency of *nauplii* (*Artemia salina*) larvae at different concentrations of DEX-PLGA MS.

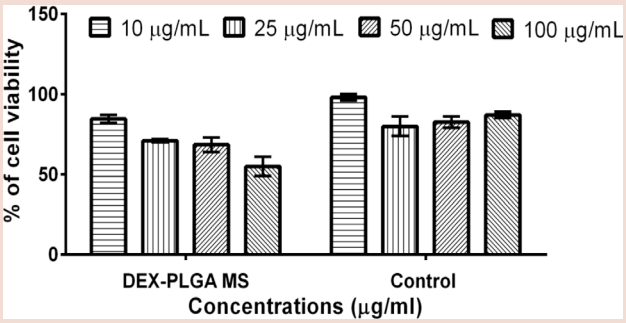

Figure 5: Evaluation of cell viability by MTT assay for DEX-PLGA MS against HEP-G2 Cell lines. The cytopathic effects (CPE) were observed with necrosis and cell damage.

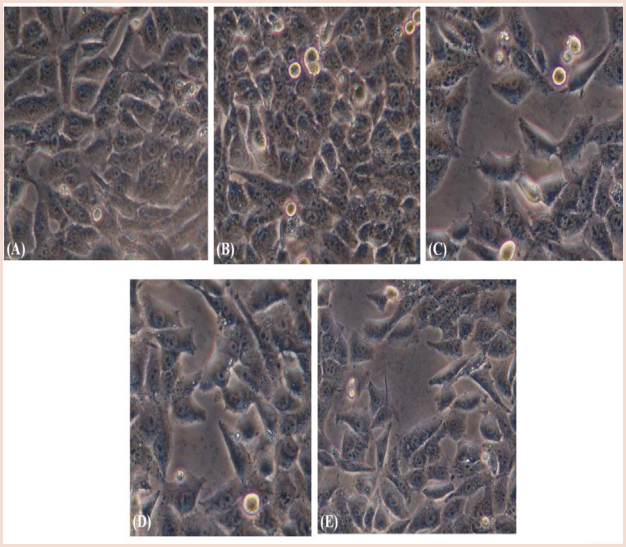

Figure 6: MTT assay for the DEX-PLGA MS with different concentrations (A) Control (B) 10 *µ*g/mL (C) 25 *µ*g/mL, (D) 50 *µ*g/mL and (E) 100 *µ*g/Ml.

slow diffusion of the DEX resulted in the prolonged cytopathic effects in HepG2 cells, therefore proving the sustained release of the DEX-PLGA MS. DEX-PLGA MS showed the growth inhibition with IC_{50} value of 3.429 mM further reduced by the addition of 50mM DEX. The plaque formation on Hep-G2 cells grown in media with 2% FBS (fetal bovine serum) with increasing order with the concentration order. MTT assay found that the free paclitaxel within the range of testing concentration and the paclitaxel reached the IC_{50} value of $6.8*10^{-6}$ M. paclitaxel-PLGA NP and paclitaxel-PLGA NP-polysorbate (PS) 80 reduced cell growth in a significant manner ($p < 0.01$, t test) by approximately 70% after 24 h of exposure. Clear dose-response relationship was observed for both PLGA NPs-paclitaxel and PLGA NPs-paclitaxel-PS 80. Decreasing the free paclitaxel concentrations from $1*10^{-4}$ M to $6.8*10^{-6}$ M resulted in a gradual decrease in the drug's cytotoxicity, and the percent of viable cells at the concentration of $6.8*10^{-6}$ M was found to be 97% showed in intact controls.

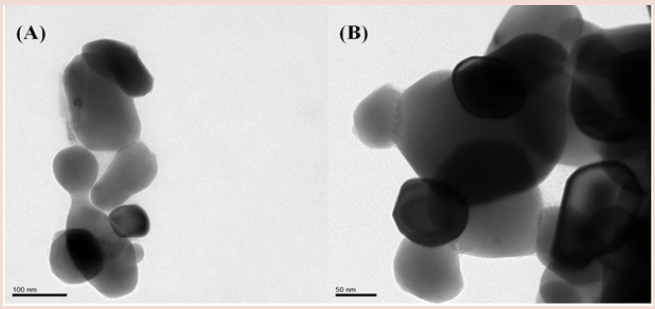

Figure 7: TEM micrograph of the DEX-PLGA MS (A) 1, 00,000 X (B) 1, 20, 000 X. It shows the spherical and oval with aggregated clusters. Size range in 80-100nm.

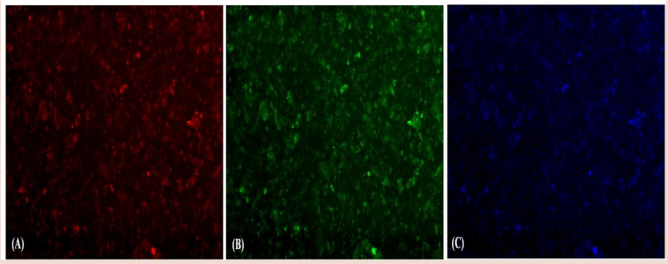

Figure 8: Confocal laser scanning microscopy studies for DEX-PLGA MS. The sustained release of DEX-PLGA MS with specific florescence emission under (A) red (B) green and (C) blue lasers.

Transmission electron microscopy (TEM)

TEM of the DEX-PLGA MS is shown in the Figure 7 A and B with magnification of 1, 00,000 X and 1, 20, 000 X, respectively revealed the oval and spherical shaped DEX-PLGA MS particles. Some of the particles were distorted in shape because of the lyophilization process occurred. The size ranges of the encapsulated microsphere were between 80 and 100 nm in size. The aggregation may have occurred due to the presence of polyvinyl alcohol (PVA) present on the surface of the microspheres which was due to the Van der Waals force, and H-H bond formation. In the present study, the TEM analysis of DEX-PLGA MS were found to be spherical, oval with an average size of 90 nm.

Confocal laser scanning microscopy (CLSM) studies

In the present study, the distribution of drugs within the microspheres was examined via confocal laser scanning microscopy as depicted in Figure 8 A, B and C. DEX appears to be distributed preferentially to the surface of the DEX-PLGA MS. The *p*-nitroaniline distributed more evenly throughout the smallest microspheres, but was localized increasingly toward the microsphere surface as the size increased. Similarly, DEX distributed to the microsphere surface maximum in large MS and was rather evenly distributed in the DEX-PLGA MS. The grainy appearance of the fluorescence may be the indicative of the larger drug crystals present in the DEX-PLGA MS (vide infra). The fluorescence of the drug loading capacity of the microspheres was shown to be at specific places indicating the targeting capability of the DEX-PLGA MS.

DISCUSSION

In the present study, no crystalline DEX was detected, and may be suggested the presence of DEX dispersed at molecular state within the PLGA MS as earlier reported.[11] 5-Fluorouracil (5-FU) a pyrimidine the diffractograms exhibited an intense peak at 2θ values near 28° due to its crystalline nature, while vacant as well as drug-entrapped nanoparticle formulations showed no characteristic peak of 5-FU.[23] Earlier researcher find out the XRD pattern of the encapsulated PLGA nanoparticles were exhibited less intensity of the diffraction peak when compared with simvastatin clearly indicated the reduction in the crystallinity of the precipitated simvastatin nanoparticles.[24] In the present study, the FTIR peaks revealed splitting and broadening of bands were noted at 2903 cm^{-1}, arising from -CH stretching of DEX-PLGA MS in the nanoparticles. The stretching peaks were markedly decreased after molecule exchange with new band appearing at 1754 cm^{-1} which was possibly indicative of C-O-C stretching in PLGA. Khaled *et al.*[18] reported the FTIR spectra showed that all the characteristic peaks of the blank MS appear clearly in the spectrum of dexamethasonye drug-loaded MS. The RP-HPLC resultant was found to be corresponds retention time of DEX drug. The resultant factor was 100%, which means that the actual loaded amount of paclitaxel was detected.[25] [F]The *in vitro* drug release of these PLGA nanoparticles/ microparticles composites shows sustained release of dexamethasone phosphate over a period of 700 h with almost no initial burst release.[26] Nanoparticles demonstrated sustained drug release for doxorubicin and verapamil, drug release was near zero-order (45 and 60% released, respectively) during the first 15 days, followed by a more sustained drug release, with about 60–70% of the entrapped drug released over a 28-day period.[27] The present study shows the DEX-PLGA MS with MTT assay using HEP-G2 showed increase in plaque formation (cytopathic effects) compared with earlier author reports. In the cytotoxic activity, cells were incubated with concentrations of 0.01, 0.1, 1 and 10 µg/ml of both pure drug and paclitaxel loaded in PLGA nanoparticles and was evaluated by assessing cell viability by the MTT assay using the BT 549 cancer cell lines.[28] The present result, TEM microphotographs obtained with the paclitaxel-loaded PLGA NPs were showed the spherical shape and homogeneous particle size distribution. Similarly, Averineni *et al.*[29] reported the paclitaxel loaded PLGA nanoparticles were found to be spherical in shape with nanosize range from 40.77 to 69.92 nm. Lin *et al.*[30] reported the doxorubicin loaded PLGA microparticles showed the positions 5-8 μm below the apical surface of the Glioma C6 cancer cells monolayer and the depths of 1 μm thick, the microparticles were actually particles internalized by the cells and not those bound to the Glioma C6 cancer cell surface. In the present study, the sustained release of the DEX-PLGA MS is reported using the confocal microscopy under blue, green and red laser emissions giving drug release. Encapsulated chelidonine in biodegradable PLGA polymers with confocal microscopy confirmed the expression and localization of caspase-3 in very little amount at the intra-cytoplasmic region, which was gradually increased after both free chelidonine and nano-chelidonine treatments of HepG2 cells. The present results revealed the polymers (PLGA) were used in different combination with the aim of obtaining a device with improved drug release characteristics and enhanced cytotoxicity against cancer cells. DEX-PLGA MS were prepared using o/w single-emulsion solvent evaporation method and characterized *in vitro*.

CONCLUSION

The controlled and sustained drug delivery system might be helpful for improving the local treatment of tumors/ cancers for longer period of time. In terms of *in vitro* drug release performance, addition of PVA delayed the release of DEX as compared with pure PLGA polymeric particles and more than 70% of the drug was still released eventually within 1 h. The MTT assay showed that although there was a greater cellular uptake of DEX-PLGA MS the new formulation showed lower initial burst and slower *in vitro* release resulted in a lower short-term cytotoxicity against HEP-G2 cells. We can conclude that there is large scope for improving the use of dexamethasone in hypertensive treatments through nanoparticle as a drug delivery system. However, despite the results obtained, due to cell culture limitation, long-term reproductive survival of tumor can only be determined using further *in vivo* animal tests.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS USED

NPs: Nanoparticles; **KBr:** Potassium bromide; **PS:** polysorbate; **DEX:** Dexamethasone; **PLGA:** Poly (lactic-co-glycolic acid); **MS:** microsphere; **XRD:** X-ray diffraction; **FTIR:** Fourier transform infrared; **RP-HPLC:** Reverse phase high-performance liquid chromatography; **TEM:** Transmission electron microscopy; **CLSM:** Confocal laser scanning microscopy; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **HEP-G2:** human liver cancer cell line; **FDA:** Food and Drug Administration; **O/W:** oil-in-water; **PBS:** Phosphate buffered saline; **PVA:** Poly vinyl alcohol; **PEG:** Poly ethylene glycol; **DCM:** Dichloromethane; **EE %:** encapsulation efficiency percent; **BST:** Brine shrimp; **WSW:** Swollen weight; **ESW:** Swelling ratio.

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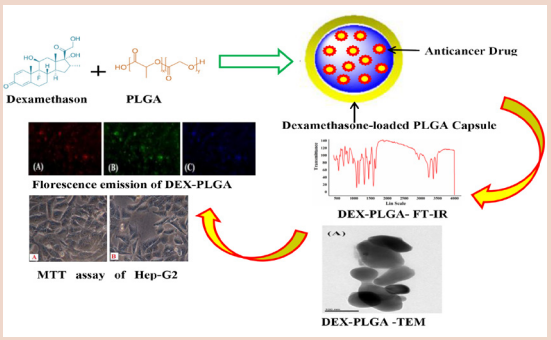
PICTORIAL ABSTRACT



SUMMARY

The polymers (PLGA) were used in different combination drug release characteristics and enhanced cytotoxicity against cancer cells.

The controlled and sustained drug delivery system might be helpful for improving the local treatment of tumours/ cancers for longer period of time.

The *in vitro* drug release performance, addition of PVA delayed the release of DEX as compared with pure PLGA.

The 70% of PLGA encapsulated drug was released eventually within 1 h.

We detected new formulation of DEX-PLGA MS displayed lower initial burst and slower *in vitro* release resulted in lower short term cytotoxicity against HEP-G2 cells.

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