FABRICATION OF POLY(DL-LACTIC-CO-GLYCOLIC ACID) NANOPARTICLES FOR ANTI-CANCER DRUG DELIVERY

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Introduction

Cancer is a group of diseases in which normal cells are converted to cells capable of autonomous growth and invasion. In the chemotherapeutic control of cancer, drugs are usually given systemically and are distributed to all parts of the body by blood causing serious side effects.

Doxorubicin (Dox) occupies a central position in the treatment of breast cancer. However doxorubicin induced cardiac toxicity is associated with a high incidence of morbidity and mortality [Gianni L. *et al*,2004, Weinberg LE *et al*.,1987]. One approach to overcome Dox-related toxicity is to use polymeric drug carriers, which direct the Dox away from sites with tight capillary junctions such as the heart muscle, and allow usage of lower dosages.

Poly(d,l-lactide-co-glycolide) (PLGA) has been used over 40 years as a biodegradable and biocompatible implant material [M. Vert *et al*,1990]. PLGA degrades by bulk degradation, and it is used for controlled release of durgs .

In the present study, Dox is encapsulated in PLGA nanoparticles by single (o/w) and double microemulsion (w/o/w) solvent evaporation techniques. In order to optimize the process, different formulations are prepared and investigated for their encapsulation efficiency, size distribution, and zeta potential.

Experimental

Materials

PLGA (75:25 ,MW66,000-107,000), polyvinyl alcohol (PVA) (MW: 30.000-70.000) are obtained from Sigma-Aldrich. Cell culture reagents and XTT cell proliferation kit were purchased from Biochrom Ag. and Biological Industries respectively. MCF-7 monolayer human epithelial breast adenocarcinoma cell line was provided from Food and Mouth Disease Institute (Şap). Doxorubicin.HCl was donated by Prof. Dr. Fikret Arpacı and Prof. Dr. Ali Ugur Ural, Gülhane Military Medical School Hospital (Ankara).

Fabrication of nanoparticles

For the preparation of PLGA nanoparticles by w/o/w double emulsion (DE) solvent evaporation method; Dox is dissolved in distilled water; and PLGA is dissolved in chloroform. Aqueous Dox solution is added into the oil phase and sonicated for 45 sec on ice .This makes the primary emulsion. To prepare nanoparticles with w/o single emulsion (SE) solvent evaporation method, Dox was solubilized in chloroform (2mg/ml) containing 5M equivalent of triethylamine with respect to Dox by sonication at 2W for 5min. PLGA is dissolved in this solution to obtain the oil phase. Then the emulsions from both methods were added into the 2% PVA solution in a closed container under constant magnetic stirring at 1400 rpm. After 10 min solvent was evaporated completely by stirring at 250 rpm. The nanoparticles were obtained by centrifugation at 15000 rpm for 15 min at 4°C. Particles were washed 3 times with distilled water to remove any residual free drugs and free surfactants, then lyophilized overnight, and kept at -80°C until use. Parameters that control the quality of particles are changed to examine the individual effects. The different formulations are given at Table 1.

Method - Label	Dox(mg)	PLGA(mg/ml)	TL %
DE - 1	0.26	25	1
DE - 2	0.52	25	2
DE - 3	1.32	25	5
DE - 4	0.53	10	1
DE - 5	0.53	30	1
DE - 6	0.53	75	1
SE - 1	0.53	4	5
SE - 2	1.11	4	10
SE - 3	1.76	4	15
SE - 4	2.5	4	20
SE - 5	2.5	8	20

Table1: Different Nanoparticle Formulations.
% TL (Theoretical Drug Loading) = drug amount / PLGA amount+drug amount *100

Characterization of Nanoparticles

To determine drug encapsulation efficiencies (EE) Dox loaded and empty particles (to be used as a blank) were weighted in triplicate and dissolved in DMSO. Samples are sonicated until the solution became clear. Absorption of the solution is measured in at 480nm. Standards are prepared from known concentrations of Dox in DMSO. The particle size and surface charge were determined by using dynamic laser light scattering technique. Nanospheres were suspended in water and sonicated for 5min prior to particle size determination. For cell viability assay, MCF-7 cells were seeded in a 96-well plate at a density of 5000 cells/well. After 24 h incubation allowing for cell attachment, the medium was removed and the cells were incubated with empty and Dox-loaded PLGA nanoparticle suspension at different concentrations. After 72h XTT assay was performed and the absorbance intensity was measured by the ELISA reader at 500 nm.

Results and Discussion

Effects of polymer concentration and drug loading on EE on size and zeta potential

Formulation	EE%	Formulation	EE%
DE - 1	20.21	SE - 1	15.49
DE - 2	14.92	SE - 2	45.69
DE - 3	10.74	SE - 3	63.32
DE - 4	15.12	SE - 4	71.53
DE - 5	25.09	SE - 5	72.33
DE - 6	40.00		

Table 2: Effects of polymer concentration and drug loading on EE% (Encapsulation efficiency = weight of drug initially used / weight of drug encapsulated * 100)

In DE method, the increase in TL from %1 to %5 resulted in a decrease of EE from %20.2 to %10.7. However, in SE method, EE increased at higher TL values. Changing TL from %10 to %20 increased EE from %15.1 to %71.5. The low entrapment of Dox in DE method could be explained by preferential localization of drug at the outer aqueous phase rather than inside the nanoparticle core, which was less hydrophilic. On the other hand in SE method, Dox is solublized in chloroform which is immiscible with water, so its escape to the outer phase is minimized. This could be the reason for higer EE values obtained in the latter method.

Figure 1 shows the effect of polymer concentration in the organic phase on the size distribution of particles prepared by DE solvent evaporation method. The increase in polymer concentration from 10mg/ml to 30mg/ml resulted in limited change on the particle size. However, when the PLGA concentration is increased to 75mg/ml two peaks are formed one having smaller particles and another one having very large particles. Large particles might have resulted from poor polymer dispersion into water due to high viscosity of the organic phase. However they can be filtered away easily.

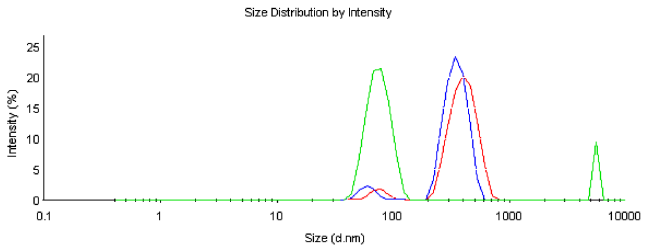

Figure 1. Size distribution graphs. Red, Blue, Green corresponds to DE-4, DE-5,DE-6 respectively.

Due to the limitations of Dox solubility in chloroform, PLGA concentration was from 4mg/ml to a maximum of 8mg/ml in particles prepared by SE method. This increase did not cause a significant change in size however , it increased the zeta potential (Table 3).

There is a profound change in zeta potential between different methods. SE method produced particles with lower zeta values.

Formulation	Mean Size (nm)	Zeta (mV)
DE - 4	(%93) 445.6±52.2	-31 ±0.98
DE - 5	(%90) 393.8±25.9	-28.8 ±3.0
DE - 6	(%88) 81.8±32.0	-32.5 ±2.54
SE - 4	(%100) 474.7±18.0	-1.6 ±1.5
SE - 5	(%100) 395.7±92.2	-10.5 ±0.28

Table 3. Mean size and Zeta Potential

XTT assays showed that the drug is released from the nanoparticles and effective against MCF-7 breast cancer cell line. Due to higher EE smaller amounts of SE-NPs are sufficient to exert the toxic effect.

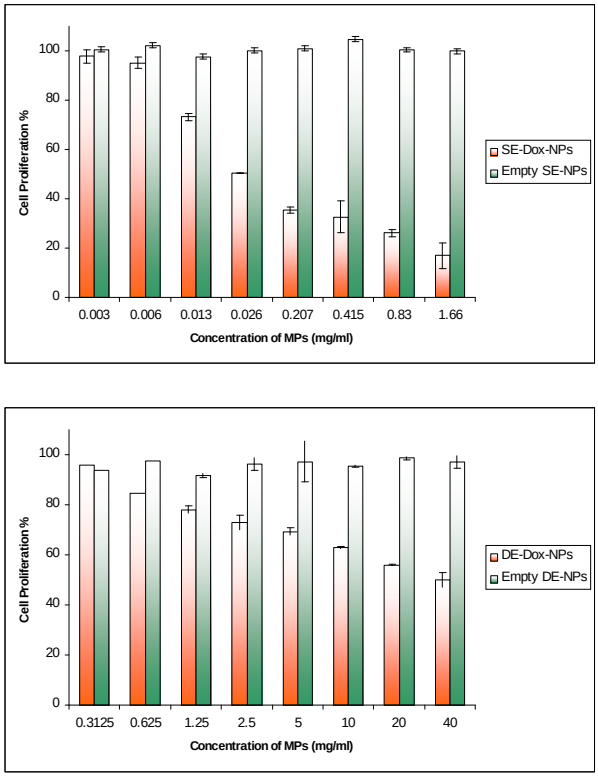

Figure 2.Toxicity of encapsulated Dox on MCF-7 cells

Conclusion

Biodegradable polymeric nanoparticles were successfully produced by w/o/w and o/w emulsion solvent evaporation methods. The results demonstrated that the o/w single emulsion technique was better to prepare Dox-loaded PLGA nanoparticles. Processing parameters are optimized to obtain highest EE and optimum size distribution. Increasing the PLGA concentration leads to higher EE values in both of the methods investigated. Dox was effective when encapsulated into the nanoparticles and exerted significant toxicity on MCF-7 cells.