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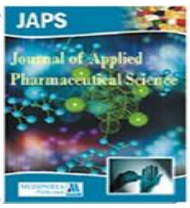

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HER-2 targeted immunonanoparticles for breast cancer chemotherapy

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ABSTRACT

The success of anti-cancer drug targeting is depends on the ability of the therapeutics to reach their desirable cellular and intracellular sites and minimizing action at the nonspecific sites. In the present study, anti-human epidermal growth factor receptor (HER-2, ErbB2) antibody anchored nanoparticles were prepared and evaluated for the assessment of targeting potential in breast cancer cell. In an attempt for comparison of carrier system for site-selective delivery, docetaxel loaded PLGA nanoparticles, PLGA-PEG nanoparticles and PLGA-PEG immunonanoparticles capable of targeting breast cancer were prepared by emulsion solvent evaporation technique. The drug-loaded nanoparticles were characterized for their size and size distribution, surface charge, drug encapsulation efficiency and in vitro drug release. Our results demonstrate that docetaxel loaded PLGA-PEG immunonanoparticles strongly enhances the site specific uptake and high cytotoxic effect at targeted sites, as compared with PLGA, PLGA-PEG nanoparticles. In conclusion polymeric immunonanoparticles could be a promising carrier for the treatment of HER2-overexpressing breast cancers.

Key words: Nanoparticles, Immunonanoparticle, Anti-HER-2 antibody, breast cancer, Docetaxel.

INTRODUCTION

The nanoparticles made from biodegradable polymers have attracted great interest for sustained drug release with reduced systemic side effects (Feng and Chien, 2003; Mu and Feng, 2003). They can passively accumulate in tumors through a mechanism known as the enhanced permeation and retention (EPR) effect (Allen, 2004). Recently, polymeric nanoparticles have been utilized for drug delivery to breast cancer. They provide better accumulation in pathological areas with compromised vasculature and targeting at desired sites with the help of specific binding ligands on the surface of nanoparticles (Kohli et al., 2007). The ideal drug for selectively targeting breast cancer is not available at a therapeutically relevant level and in clinical practice. Docetaxel is a very potent anticancer agent and used in treatment of various types of cancer but the currently available marketed formulation of docetaxel produce acute hypersensitivity reactions in the majority of patients treated in phase I clinical trials (Rowinsky, 1997; Capri et al., 1996; Kaye, 1995). The functionalized nanoparticles with specific binding ligands on their surface can be exploited for targeted delivery of drugs, therapeutics, and diagnostics of cancer (Chen, 2008). Development of monoclonal antibodies (mAb) and its utilization for targeting can improve the efficacy of the active molecule, improving distribution and concentration of the drug at the site of injury/disease (Trail and Bianchi, 1999; Funaro et al., 2000; Garnett, 2001; Trail et al., 2003; Lambert, 2005; Kocbek et al., 2007). Antibodies are well-established systems for targeting drug loaded colloidal carriers to specific cells or receptors (Steinhauser, 2006). Targeted drug delivery can be obtained by functionalization antibody with the drug-loaded nanoparticles. Tumor targeting

specific antibodies have been devised to initiate specific signalling cascades which can potentiate the therapeutic effect of attached drug (Fahmy, 2005; Sapra, 2003; Sun et al., 2008).

Many studies utilized HER-2 (Human Epidermal Growth Factor Receptor) receptor for HER-2 positive breast cancer (observed in 20-25% of all breast cancer patients). HER-2 is a member of tyrosine specific protein kinase family consists of four EGF receptors i.e. EGFR (ErbB1), ErbB2 (HER-2), ErbB3, and ErbB4 (Baulida et al., 1996; Frankel, 2002; Nair, 2005; Widakowich et al., 2007) which contain a cytoplasmic tyrosine kinase domain, a single transmembrane domain, and an extracellular domain that is involved in ligand binding and receptor dimerisation (Slamon, 1987). An overexpression of HER-2 at breast cell surface may lead to potentiate dysregulated growth, angiogenesis, metastasis and resistance against apoptosis-inducing therapeutic agents (Harari and Yarden, 2000). In present study, HER-2 specific monoclonal antibody anchored immunonanoparticles were developed and the targeting potential of developed formulation was compared with plain PLGA and pegylated PLGA nanoparticles. Covalent conjugation method was employed to attach mAb to the surface of pre-formed PLGA-PEG nanoparticles with a drug.

MATERIALS AND METHODS

Materials

PLGA with free end carboxyl groups (50:50, MWt. 40,000-75,000 Da), PEG diamine (average MWt. 3,350 Da, Purity >95 %), Dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), anti-HER-2 mAb, were obtained from Sigma, USA; Docetaxel was kindly supplied by Cipla, India; Sodium cholate (99 %) was procured from otto, India; Dichloromethane (DCM) was purchased from Rankem, India. All other reagents and solvents were of analytical grade.

Synthesis of PLGA-PEG diblock copolymer

PLGA-PEG diblock copolymer was synthesized by DCC and NHS chemistry (Yoon et al., 2002). The PLGA (100 mg) was dissolved in DCM (20 ml). After that DCC (0.24 g) and NHS (0.13 g) were added under magnetic stirring. The activation reaction of carboxylic acid end group of PLGA was continued by 12 h magnetic stirring at room temperature. Insoluble dicyclohexyl urea was filtered and the resultant solution was precipitated by dropping into ice cold anhydrous diethyl ether. The activated PLGA was completely dried under vacuum. The NHS ester of PLGA was conjugated with PEG diamine via amide linkage. The activated PLGA (100 mg) was dissolved in DCM (20 ml) and then excess of PEG diamine (100 mg) was added. The coupling reaction was performed under magnetic stirring for 9 h at room temperature. An excess amount of PEG diamine was used to prevent the formation of PLGA-PEG-PLGA triblock copolymers. The amine terminated PEG-PLGA diblock copolymer was precipitated with cold methanol and purified with excess of ethanol (to eliminate unconjugated PEG diamine) and finally dried under vacuum.

Preparation of PLGA and PLGA-PEG nanoparticles

The nanoparticles were prepared by the o/w emulsification solvent evaporation method (Senthilkumar *et al.*, 2007). Initially, 100 mg of copolymer was dissolved with or without drug in 2 ml of DCM. Then it was vortexed and emulsified in 20 ml of a 0.1% sodium cholate solution in a sonicator (ultrasonic cell crusher, classic, Lark Innovative Fine Teknowledge, Chennai, India) at 20W output for 2 min. The organic solvent was evaporated at room temperature. Subsequently, nanoparticles were recovered by centrifugation 20,000 x g, 20 min, 4°C), washed trice with water and lyophilized. The dried nanoparticles were stored in the refrigerator at 4°C. Each sample was prepared in triplicate.

Preparation of PLGA-PEG immunonanoparticles

The immunonanoparticles was prepared by slightly modifications in reported method given by Steinhauser et al., 2006. The preparation of immunonanoparticles includes three steps:

1. Activation of NH_2 group of PLGA-PEG nanoparticles with MBS.
2. Thiolation of anti-HER-2 monoclonal antibody.
3. Conjugation of thiolated anti-HER-2 monoclonal antibody on the surface of MBS activated nanoparticles.

In the first step, PLGA- PEG nanoparticles (5 mg) was dispersed in 1 ml PBS (pH7.2) and incubated with 100 µl crosslinker MBS solution (3.14 mg MBS dissolved in 1 ml of DMSO) for 30 min at 25 °C temperature in shaker incubator. The excess of MBS was separated from activated nanoparticles using sephadex G25. Secondly, for thiolation of anti-HER-2 mAb, 5.7 mg of 2-iminothiolane (Traut's Reagent) was dissolved in 5 ml PBS (pH 7.4) and 100 µl anti HER-2 mAb was incubated with 4.2 µl of 2-iminothiolane for 2 hr at 20 °C under constant shaking. Finally, 100 µl thiolated mAb was added in 100 µl MBS-activated nanoparticles for 30 min at 25 °C under constant shaking for preparation of PLGA-PEG immunonanoparticles.

Characterization of nanoparticles and immunonanoparticles

Particle size and size distribution

The particle size was determined by dynamic light scattering (DLS) (Zetasizer 4000, Malvern Instruments Ltd., Malvern, UK) at 25 °C at an angle of 90°, taking the average of three measurements. The particle dispersion was diluted with water to such a degree that the desired number of counts was obtained. The desired number of counts is the number of counts that is high enough to get the highest possible signal to noise ratio, yet small enough to prevent multiple scattering to occur.

Surface morphology

To examine the shape and morphology of the nanoparticles, samples were analyzed using Scanning Electron Microscopy (SEM). The nanoparticles were placed on sample holder, coated with gold-palladium alloy (150-250Å) using a sputter coater and then placed in scanning electron microscope.

Drug entrapment efficiency

The amount of docetaxel entrapped in nanoparticles and immunonanoparticles was measured by UV spectrophotometer using a detection wavelength of 229.5 nm. The percentage drug entrapment was measured as:

$$\% \text{ DEE} = \frac{\text{Amount of drug entrapped}}{\text{Amount of drug added}} \times 100$$

The individual values for triplicate determinations and their mean values were reported.

Zeta potential measurements

Zeta-potential measurements by laser Doppler electrophoresis were performed on particles redispersed in 10 mM NaCl at the same concentration as used for particle analysis (Zetasizer 2000, Malvern Instruments Ltd., Malvern, UK), taking the average of three measurements.

In vitro drug release

To perform the in vitro release study the nanoparticles were suspended in PBS (pH 7.4). Vials containing 10 mg of nanoparticles and 10 ml of PBS were incubated at 37°C on a constant shaking mixer (LabTech, LSI-2005RL). At appropriate time intervals 1.0 ml of release medium was collected following centrifugation at 22,000×g for 30 min and 1.0 ml of fresh PBS (pH 7.4) was again added to the vial. The amount of drug (docetaxel) was determined by measuring the drug concentration in the supernatant. To determine the drug content in the supernatant, 1ml DCM was added to extract docetaxel. A nitrogen stream was introduced to evaporate DCM and 2 ml of methanol were added to dissolve the drug. The clear solution was analyzed by UV as previously described under drug entrapment studies. The release profiles for each preparation were determined in triplicate.

SDS-PAGE (SDS Polyacrylamide Gel Electrophoresis)

The primary structural integrity of anti-HER-2 antibody after conjugated onto the NP surface was detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in comparison with the native HER2 antibody and reference markers. All the gels were run under reducing conditions by using Mini-Protean II electrophoresis unit from BioRad at a constant voltage mode of 200V in a Tris/glycine/SDS buffer. The gels were stained with silver to reveal protein, destained and dried.

Cell culture

HER-2 positive MCF-7 cells were cultured in DMEM (Sigma chemicals, USA) supplemented with 10% inactivated new born calf serum, penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphoterecin-B (5 µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in phosphate buffer saline solution, the stock culture was grown in 25 cm^2 tissue culture flasks and all experiments were carried out in 96-well microtitre plates.

Ex-vivo cellular uptake studies

HER-2 positive MCF-7 cells were seeded in 96-well microtiter plates. After the cells reached 80% confluency, they were incubated with suspension of DAPI loaded PLGA nanoparticles, PLGA-PEG nanoparticles and PLGA-PEG immunonanoparticles at 37 °C at the concentration of 0.25 mg/ml for 4 hr. At the end of the incubation period, the nanoparticles suspension was removed from the wells and the cell monolayers were rinsed three times with cold PBS (pH 7.4) to remove uninternalized nanoparticles. After removal of nanoparticles suspension and washing the cell monolayers thrice with PBS, the cells were fixed by an appropriate amount of ethanol and incubated for 20 min, followed by addition of propidium iodide to stain the nucleus after removal of ethanol. Finally, the cells were mounted by the mounting medium and observed under confocal laser scaning microscopy using a FITC filter.

Ex-vivo cytotoxicity assay

Cell lines in exponential growth phase were washed, trypsinized and resuspended in complete culture medium. Cells were plated at 1 × 104 cells/well in 96 well microtitre plate and incubated for 24 h during which a partial monolayer forms. The cells were then exposed to various concentrations of blank and docetaxel loaded NPs suspensions in quadruplicate. The inhibition of cell growth was assessed by SRB assay according to the reported method (Skehan *et. al.*, 1990). Culture plates were taken out from the incubator after 48 h incubation of cells with test material. 50µl of chilled 50% TCA was laid on the top of medium in all the wells to produce a final concentration 100%. The culture plates were incubated at 4 °C for one hour to fix the cells attached to bottom of the wells. The plates were washed with distilled water (5-6 times) to remove TCA and plates were then air-dried. 100 µl of 0.4% (w/v) solution of SRB in 1% acetic acid was added to each well of the plate and the plates were left at room temperature for 30 min. The plates were washed with 1% acetic acid after 30 min, to remove the unbound SRB. Plates were again air-dried and 100 µl of Tris buffer was added to each well. The plates were gently shaken for 10- 15 min on a mechanical shaker. The optical density (OD) was measured with ELISA reader at 540 nm wavelength & the data was maintained. The cell viability and growth in the presence of test material is calculated as:

$$\textbf{\% Cell viability} = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where, $\text{Abs}_{\text{sample}}$ was the absorbance of cells tested with various formulations and $\text{Abs}_{\text{control}}$ was the absorbance of control cells (incubated with cell culture media only).

RESULTS AND DISCUSSION

Particle size, size distribution and drug entrapment efficiency

The size and size distribution along with the drug entrapment efficiency of docetaxel loaded PLGA, PLGA-PEG nanoparticles and PLGA-PEG immunonanoparticles are listed in Table 1, which shows that PLGA-PEG immunonanoparticle exhibited a size (nm) 158.9 ± 8.85 with polydispersity 0.2730 ± 0.04. The anti-HER-2 antibody attachment slightly increases the nanoparticles size and had little effect on drug entrapment efficiency. A polydispersity index of 1 indicates large variations in particle size and of 0 indicates no variation in particle size (an ideal monodisperse formulation).

Table 1. Various parameters of PLGA and PLGA-PEG nanoparticles and PLGA-PEG immunonanoparticles.

S. No.	Parameters	PLGA nanoparticles	PLGA-PEG nanoparticles	PLGA-PEG immunonanoparticles
1	Size (nm)	122.7 ± 17.28	150.1 ± 14.32	158.9 ± 8.85
2	Polydispersity index	0.104 ± 0.03	0.217 ± 0.01	0.273 ± 0.04
3	Zeta potential (mV)	-39.7 ± 1.6	-14.1 ± 1.2	-2.3±1.2
4	% DEE	73.416 ± 1.14	72.124 ± 1.59	71.942 ± 2.03

Surface morphology

Morphology studies of PLGA and PLGA-PEG nanoparticles were performed utilizing scanning electron microscopy. The particles were observed spherical, smooth and morphologically similar (Fig. 1).

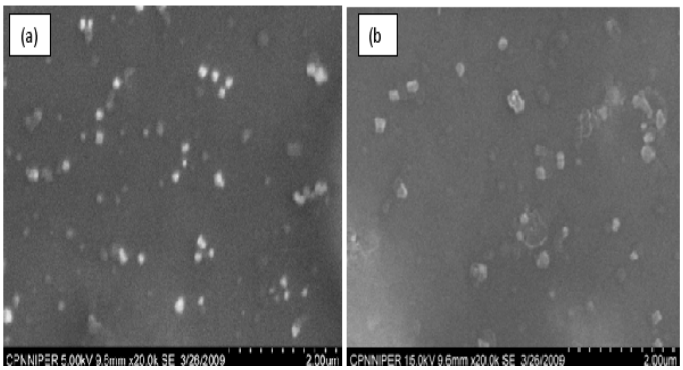

Fig 1. SEM Photographs of (a) Plain PLGA nanoparticles (b) PLGA-PEG immunonanoparticles.

Zeta potential

The zeta potential, *i.e.* the surface charge of the Docetaxel-loaded PLGA, PLGA-PEG nanoparticles and immunonanoparticles are recorded in Table 1. Zeta potential is an important factor to determine the stability of the NPs in dispersion and also plays an important role in the interaction between the cell membrane and the NPs. PLGA nanoparticles exhibited highest zeta potential (-39.7 mV ± 1.6) due to presence of free carboxyl end group on particle surface, in the case of PLGA-PEG nanoparticles the zeta potential (-14.1 mV± 1.2) found to get shifted lesser negative value due to the presence of amine terminated PEG chains, because the coating layer shields and move the shear plane outwards from the particle surface. A marked difference in the surface charge between PLGA and PLGA-PEG nanoparticles was observed. The result confirms that the amine terminated PEG chains were situated on the surface of the nanoparticles. The hydrophilic NH_2-PEG moiety was oriented outside towards aqueous medium forming corona type, which was anchored to hydrophobic PLGA polymer backbone as a core material. The value of zeta potential shifted to lesser negative because of the positive charge of anti-HER-2 antibody.

In-vitro drug release

The in vitro release profile of PLGA and PLGA-PEG nanoparticles is shown in Fig. 2.

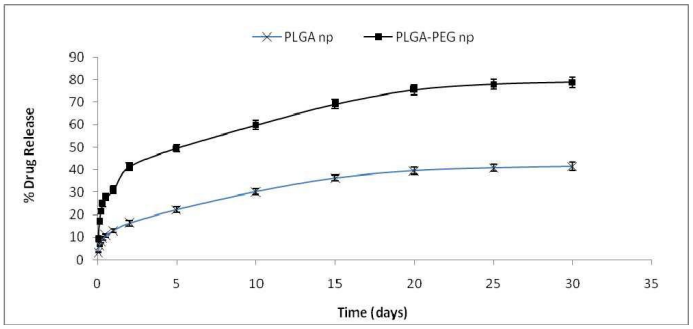

Fig 2. In- vitro release profile of docetaxel loaded nanoparticles in PBS (pH 7.4) at 37°C.

The studies were carried out in PBS (pH 7.4) at 37°C.on a constant shaking mixer. The drug release profile was studied for various time intervals i.e. 1hr, 3hr, 5hr, 8 hr, 12 hr, 24 hr, 2 day, 5 day, 10 day, 15 day, 20 day, 25 day and 30 day. Both exhibit a biphasic release pattern that was characterized by an initial burst upto 24 hr, followed by a slower sustained release and remained constant upto 30 days. At the end of 30 days, the PLGA-PEG nanoparticles showed higher release rate of drug than PLGA nanoparticles. The PLGA-PEG nanoparticles degraded quickly and released the drug subsequently. This could be explained by the degradation mechanisms; the PEG chains also enhance the water penetration in to the matrix and the NPs were degraded homogeneously over time.

In contrast, PLGA formulation released only (41.397 ± 1.90) % drug due its hydrophobicity and hence poor degradation in aqueous environment. It is inferred that the gradual degradation of the polymer resulted in sustained release of the drug. This could be explained by the degradation mechanisms; the PEG chains enhance the water penetration in to the matrix and NPs were degraded homogeneously over time. However, the release of docetaxel depends on several factors including the drug to polymer ratio, drug solubility inside the matrix, and the interaction between the core and drug.

SDS-PAGE

The integrity of anti-HER-2 antibody after conjugated on the nanoparticles surface was analyzed by SDS-PAGE (shown in Fig. 3) in comparision with the native anti-HER-2 antibody. Study revealed that antibody conjugated on nanoparticle surface was almost same in structure as the native HER-2 antibody, which also validates the feasibility of PLGA-PEG immunonanoparticles for HER-2 overexpressed cancer targeting.

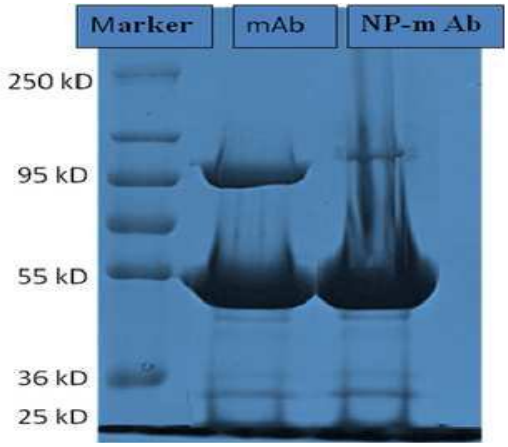

Fig 3. SDS-PAGE of anti- HER-2 antibody.

***Ex-vivo* cellular uptake studies**

Various nanoparticles formulations encapsulated with fluorescent dyes were used to study cellular uptake. DAPI, fluorescent dye was incorporated in nanoparticles as a marker for fluorescence microscopy to study the nanoparticles uptake in cells. Confocal images of the MCF- 7 cells after incubation with 0.25 mg/ml fluorescent DAPI loaded nanoparticles suspension for 4h at 37ºC are shown in Fig. 4.

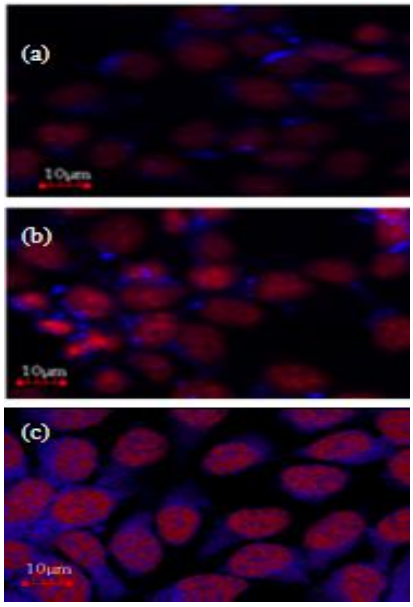

Fig 4. Confocal laser scanning microscopy (CLSM) of MCF-7 breast cancer cells after 4 h incubation with the DAPI loaded (a) PLGA Nanoparticles (b) PLGA-PEG Nanoparticles (c) PLGA-PEG immunonanoparticles at 0.25 mg/ml nanoparticle concentration at 37 ºC.

In the cell cytoplasm, around the nucleus (PI stained) are aggregates of the blue colour of DAPI loaded nanoparticles, which indicates that Nanoparticles have been internalized by the cells. It can thus be concluded that the fluorescent Nanoparticles were located inside the cells but not within the cell membrane. In the present study, the cellular uptake of PLGA-PEG immuno-nanoparticles was higher than that of PLGA and PLGA-PEG nanoparticles in HER-2 positive MCF-7 cells. As anti-HER-2 antibody modified nanoparticles were developed for a specific targeting of HER-2 receptors in breast cancer cells. The cellular uptake of PLGA-PEG nanoparticles was higher than that of PLGA nanoparticles in MCF-7 cells. The increased uptake of nanoparticles occurred via a fluid phase endocytic mechanism because there is no specific affinity between the outer PEG chains and negatively charged plasma membrane of the cells. The outer shell composed of diamine PEG could reduce the interaction between nanoparticles themselves and between nanoparticles and cells by forming a 'stealth surface' (Panyam et al., 2003). In general, the negatively charged cell membrane has a tendency to absorb positive charged or neutral nanoparticles. For PLGA-PEG nanoparticles, the zeta potential is -14.1 ± 1.2 mV. In contrast, the zeta potential for PLGA nanoparticles is -39.7 ± 1.6 mV, indicating a negative surface charge. The electronic repulsion between PLGA nanoparticles and cells resulted in fewer nanoparticles being taken up by the cells.

***Ex-vivo* cytotoxicity assay**

In cytotoxicity study, docetaxel concentrations in all the formulations were adjusted to be the same. Percentage cell viability of all formulations at different concentrations in MCF-7 cell lines was shown in Fig. 4. PLGA-PEG immunonanoparticles showed significantly higher cytotoxic effect than PLGA-PEG and PLGA nanoparticles after 48 h incubation period in MCF-7 cell lines. PLGA nanoparticles had a lower cytotoxicity at the same drug concentration. This phenomenon appeared to correspond reasonably well to the cellular uptake efficiency and drug release properties. Firstly, the cells uptake was more in PEGylated nanoparticles than non-PEGylated nanoparticles, which means that more docetaxel was engulfed by the cells. Secondly, from the *in vitro* drug release experiment, it was clearly shown that more docetaxel released from the PLGA-PEG nanoparticles than PLGA nanoparticles during 48 hrs.

This proves that the cytotoxicity of the PEGylated nanoparticles was dominated by the actual intracellular drug concentration caused by either particle cellular uptake or extra cellular drug release from the nanoparticles. PLGA-PEG immunonanoparticles exhibited highest cytotoxicity at same drug concentration. This was due to presence anti- HER-2 monoclonal antibody attached on surface of Nanoparticles for targeting HER-2 positive MCF-7 breast cancer cells, which produce a synergestic effect with anticancer drug, docetaxel. This was proved by ex-vivo cytotoxicity study of blank PLGA-PEG immunonanoparticles (without docetaxel) and docetaxel loaded PLGA-PEG immunonanoparticles (Fig. 5).

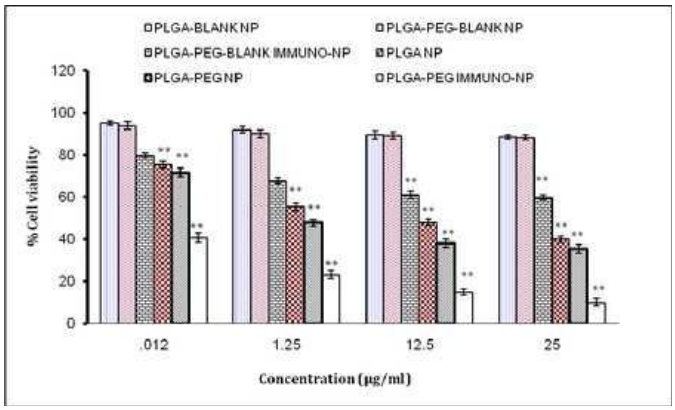

Fig 5. Ex-vivo viability of MCF-7 breast cancer cells treated with the PLGA,PLGA-PEG nanoparticles and PLGA-PEG immunonanoparticles (loaded with and without docetaxel) after 48 h. [Each data represents mean ± S.D. (n=4). Significance was tested using one way ANOVA and Dunnett post test. One asterisk represents $p<0.05$, two asterisks $p<0.01$, three asterisks $p<0.001$ compared to all formulations with blank PLGA nanoparticles].

The blank PLGA and PLGA-PEG nanoparticles exhibited low cytotoxicity in MCF-7 cell lines. It implied that these nanoparticles could be useful as drug carriers without any significant cytotoxic effects. The significant cytotoxicity was shown by antibody alone and synergestic effect was shown along with docetaxel.

DISCUSSION

Nanotechnology offers an unprecedented opportunity in rational delivery of drugs to solid tumors following systemic administration. Nano-carriers provide researchers with potential tools to surmount many of the current limitations in conventional chemotherapy, including undesirable biodistribution, cancer cell drug resistance, and severe systemic side effects. Furthermore, advances in nanotechnology and polymer chemistry should produce numerous structures that may lead to the development of nanosized drug delivery systems aimed at generating a magic bullet against all cancers. To lower cytotoxicity and increase therapeutic effects, targeted drug delivery systems for anti-tumor drugs have been developed over last few years. These drug delivery systems including polymeric carriers and colloidal carriers like liposomes, immunoliposomes, microspheres, are often directed against epitopes present on tumor cells and/or receptors expressed on tumor cells and carry drugs which interfere with tumor growth. In all cases, the bioactive has to cross the tumor blood vessel wall consisting of endothelial cells and basement membrane. Especially in drug delivery strategies in which polymeric, macromolecular or particulate carriers are used to increase treatment selectively, the endothelial barriers form a major obstacle. The focus of research shifts from the manipulation in barrier function or designing of carrier systems that can cross the tumor vasculature, to identification of recognition elements on tumor cells, which could be targeted using suitable ligands anchored on these carrier systems. Moreover, surface modification of nanoparticles with hydrophilic polymers (poly (ethylene glycol) (PEG), polaxamer and poloxamine) will not only improve blood circulation time, but also sterically stabilized against opsonization and subsequent phagocytosis (Illum and Davis, 1986; Illum et al., 1987; Porter et al., 1992; Allen, 2002; Avgoustakis, 2004). The precise and selective binding of ligand to its corresponding receptor provides tumor specificity and limited toxicity possible and may overcome obstacles presented by cytotoxic chemotherapy. Ligand anchored surface modified nano-carriers improve surface functionality which in turn makes nanocarriers intelligent and efficient delivery system (Allen, 2002; Sapra and Allen, 2003; Zhang et al., 2007). The anchoring of ligands has been achieved by chemical (covalent or noncovalent) and physical (adsorption or ionic interaction) coupling to the surface of nanocarriers. For conjugating monoclonal antibody to the surface of nanocarriers, covalent coupling is widely used technique (Nahar et al., 2006). Monoclonal antibody attached on the surface of nanocarrier provides improved specificity for targeting hence increase the therapeutic efficacy (Kohler and Milstein, 1975; Kocbeck et al., 2007). In the present study docetaxel loaded PLGA nanocarrier systems (PLGA nanoparticles, PLGA-PEG Nanoparticles and PLGA-PEG immunonanoparticles) were developed for site-specific targeted delivery to specific HER-2 receptors overexpressed on breast cancer cells. PLGA-PEG diblock copolymer was synthesized by DCC and NHS chemistry (Yoon et al., 2002) and then docetaxel loaded PLGA and PLGA-PEG nanoparticles were prepared by emulsification solvent evaporation method. Anti-HER-2 antibody anchored PLGA-PEG immunonanoparticles was prepared by direct covalent linkage of thiolated mAb with MBS containing PLGA nanopaticles. This method has proved to be very convenient for coupling antibodies to nanoparticles for targeting purposes. Studies demonstrated that PLGA-PEG nanoparticles release drug content in slower rate compared to PLGA nanoparticles. This release rate shows the potential of the nanoparticles to provide a sustained drug delivery system. Further, in-vitro uptake analysis on breast cancer cell lines demonstrated the potential of anti-HER-2 antibody attached PLGA-PEG immunonanoparticles.

CONCLUSION

Developed surface functionalized PLGA nanoparticle systems provide novel tools for effective targeted delivery of drugs into specific cells. This study demonstrated that alterations into the surface properties and composition of nanocarriers could manipulate pharmacokinetic and phamacodynamic profile of encapsulated drug. It improves the high drug concentrations to the cancer cell, with reduced toxicity of normal tissue. Our results demonstrate that docetaxel loaded PLGA-PEG immunonanoparticles strongly enhances the cytotoxic effect at targeted sites, as compared with PLGA, PLGA-PEG nanoparticles. PLGA-PEG immunonanoparticle can provide suitable tool for effective targeted delivery of docetaxel in case of HER-2 positive breast cancer therapy, since targeted delivery reduces side effects caused by unspecific drug uptake into healthy tissues.

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