International Journal of Pharmaceutics 399 (2010) 80–86

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Anti-CD8 conjugated nanoparticles to target mammalian cells expressing CD8

A. Bicho, Inês N. Peça, A.C.A. Roque, M. Margarida Cardoso*

REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

ARTICLE INFO

Article history:
Received 18 May 2010
Received in revised form 28 July 2010
Accepted 2 August 2010
Available online 7 August 2010

Keywords:
PLGA nanoparticles
Active targeting
Surface modification
Antibody anti-CD8
Drug delivery systems

ABSTRACT

This work aimed at the development of targeted drug delivery systems using nanoparticles fused with antibodies. The antibody anti-human CD8 was coupled onto PLGA nanoparticles, and the ability of these particles to specifically target cells expressing CD8 was studied. The obtained particles were found to be of spherical shape exhibiting a size between 350 and 600 nm. In vitro experiments with different cellular cultures (TE671, CHO and HEK293) using unmodified nanoparticles containing rhodamine have shown that particles were present on their surface within 48 h of incubation. In vitro tests using anti-CD8 conjugated nanoparticles in CHO cell cultures indicated that all transfected cells which express CD8 show these particles on their surface within 1 h of incubation. These results demonstrated that, in a shorter time, the produced particles can target cells expressing CD8 on their surface which offers the ability to reduce drug side effects. The antibody-coupled nanoparticles represent a promising approach to improve the efficacy of active targeting for lymphoblastic leukaemia therapy.

1. Introduction

One of the greatest problems for patients submitted to cancer therapies is the unspecificity of effects that afflicts many organs and causes severe secondary side effects. In particular, chemotherapy greatly damages healthy tissues in the process of killing cancer cells (Brannon-Peppas and Blanchette, 2004; Liu et al., 2007). Methods which may offer an increase of the local drug concentration at the tumor, while lowering the systemic dose, offer the ability to kill only cancer cells and to affect as few healthy cells as possible (Scott et al., 2008).

Active drug targeting represents a promising approach for cancer treatment when specific expression of particular target antigens, receptors and biomarkers can be exploited. The delivery of drugs to cancer cells can be selectively increased by associating the drugs with molecules that bind to antigens or receptors that are either uniquely expressed or overexpressed on the target cells relative to normal tissues (Allen, 2002). Direct conjugation of a drug molecule to a recognizing unit is possible but the reduced number of drug molecules that can be attached as well as the possible lack of pharmacological activity due to the coupling reaction can be limiting factors for such a strategy (Allen, 2002). Polymeric nanoparticles with specific recognition ligands bound to the surface represent an attractive approach for site-selective delivering of drugs (Liu et al., 2007). Besides the capacity to improve the drug therapeutic index the drug targeting by nanoparticles may combine the advantages of drug carrier systems: high drug loading capacity, controlled release, delivery of hydrophobic drugs and increase of drug stability (Fahmy et al., 2005a, 2005b; Sahoo and Labhasetwar, 2003). Several in vitro studies have explored the possibility of using antigen/antibody systems (Dinauer et al., 2005; Kocbek et al., 2007; Obermajer et al., 2007; Scott et al., 2008; Tobío et al., 1998; Yang et al., 2007) or making use of other molecular recognition mechanisms such as antigen protein/RNA aptamer (Cheng et al., 2007; Farokhzad et al., 2004, 2006). Biodegradable nanoparticles have been extensively investigated for sustained and targeted/localized delivery of different agents including plasmid DNA (Bivas-Benita et al., 2004; Csaba et al., 2006; Prabha et al., 2002), proteins (Cegnar et al., 2004; Coester et al., 2006; Davda and Labhasetwar, 2002; Li et al., 2001; Panyam et al., 2003) and peptides (Clawson et al., 2010) and low molecular weight compounds (Panyam and Labhasetwar, 2003).

Polymers such as poly(D,L-lactide-co-glycolide) (PLGA), already approved by FDA, have been used in drug delivery applications due to their biodegradability, biocompatibility (Bivas-Benita et al., 2004; Panyam and Labhasetwar, 2003) and to their great flexibility with respect to the manipulation of physicochemical properties of the polymer and the range of antigens and immunomodulators that they can accommodate (Hamdy et al., 2008).

Within this context, the aim of this study was to investigate the possibility of directing PLGA nanoparticles to target cells expressing the human CD8 membrane protein, which is a recognized cellular marker of lymphoblastic leukaemia cells (Jurman et al., 1994; Pui and Jeha, 2007), by means of functionalizing the particles with the anti-human CD8 antibody. We examined whether the targeting and uptake of these vehicles by CD8 expressing cells could be achieved

* Corresponding author. Tel.: +351 21 2948385; fax: +351 21 2948385.
E-mail address: margarida.cardoso@dq.fct.unl.pt (M.M. Cardoso).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.08.005

by following the unspecific internalization of the PLGA particles and the specific internalization of antibody anti-human CD8 conjugated PLGA particles by different mammalian cell lines over a period of four days. We show that it is possible to specifically direct PLGA particles functionalized with the anti-human CD8 antibody to cells expressing CD8 at their membrane at shorter periods (1 h) when comparing with non-functionalized particles.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA) (50:50) (Mw 40,000–75,000), poly(vinyl alcohol) (Mw 30,000–70,000) (PVA), rhodamine B 95% purity, rabbit anti-goat IgG (FITC conjugated), the anti-human CD8 IgG (R-phycoerythrin conjugated) and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (99.5% purity) were purchased from Sigma. Dichloromethane (DCM), (99.9% purity), acetone (95% purity) and phosphate buffered saline (PBS) were bought from Fluka. 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHS) were obtained from Aldrich. Glycine 99.7% purity was bought from Rieldel-de Haen. All cell culture disposable materials are from Sarstedt. Culture media, foetal bovine serum, trypsin, antibiotics and fungizone were purchased from Gibco. CD8 or GFP cDNA subcloned into the pIRES plasmid (CLONTECH) were a kind gift by Dr. Erwan Michard (Jurman et al., 1994). FuGENE 6 transfection Reagent was obtained from Roche. Dynabeads are from Dynal® Biotech. EDTA (99.5% purity) was obtained from AnalaR.

2.2. Methods

2.2.1. Preparation of PLGA nanoparticles (NP)

PLGA nanoparticles (NP) were prepared using an oil-in-water emulsion solvent extraction/evaporation technique: 50 mg of the PLGA dissolved in 500 μl of acetone and 750 μl of DCM. The polymer solution was added to 10 ml of an aqueous solution containing 3% (w/v) PVA as a stabilizer. The mixture was emulsified for 20 s with a sonicator operated at 70 W. The formed o/w emulsion was poured into 50 ml of a PVA aqueous solution (0.25%, w/v) and magnetically stirred for 24 h at room temperature to completely extract/evaporate the organic solvent and harden the particles. The produced nanoparticles were collected by centrifugation at 11,000 rpm (Sartorius, Sgma 4K15), washed three times with deionised water and freeze-dried (Telstar Cryodos).

PLGA nanoparticles containing rhodamine (RNP) as a particle fluorescence marker were prepared in the same manner adding rhodamine to the polymer solution.

2.2.2. Characterization of nanoparticles

2.2.2.1. Surface morphology. Scanning electron microscopy (SEM) was utilized for the characterization of the surface morphology and estimation of the size of the produced nanoparticles. Before observation, powdered samples of freeze-dried nanoparticles were fixed onto metallic studs with double-sided conductive tape and coated with gold under argon atmosphere. A DSM 962 (Zeiss, Germany) scanning electron microscope was used with an accelerating voltage of 20 kV.

2.2.2.2. Particles size and zeta potential. The mean particle diameter and the zeta potential of the produced nanoparticles were measured by dynamic light scattering (DLS) performed in a Zetasiser Nano Series ZEN3600 (Malvern, UK) at 298 K operating with a 633 nm laser. The detection uses a non-invasive back scattering technique (173°). Freeze-dried nanoparticles were dispersed in an aqueous solution of PVA 0.50% (w/v), sonicated, washed three times with deionised water and appropriately diluted before each measurement. All measurements were made in triplicate.

2.2.2.3. Fourier transformed infrared spectroscopy (FTIR). Fourier transformed infrared spectroscopy (FTIR) was used to confirm the characteristic bands of PLGA nanoparticles and PLGA nanoparticles containing rhodamine. FTIR spectra were recorded on a Shimadzu FTIR spectrometer using high sensitivity pyroelectric detector (DLATGS). Pressed pellets of dried nanoparticles were prepared by grinding the samples with KBr in a mortar with 1:100 ratios. The tablet recovered, was immediately analyzed in the region of 400–4000 cm^{-1}.

2.2.3. Coupling of the anti-human CD8 antibody to the nanoparticles (anti-CD8–RNP)

A suspension of 1.5 mg of particles containing rhodamine (RNP) in 250 μl of 1 M HEPES 0.5 M NaCl (pH 8) was incubated with 2 equivalents of 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and 2 equivalents of N-hydroxysulfosuccinimide (NHS) for 15 min at room temperature with gentle stirring. The NHS-activated particles were incubated with 100 μl of IgG Anti-Human CD8 for 2 h in darkness at room temperature in a 200 rpm orbital incubator. The resulting nanoparticles–anti-CD8 bioconjugates (anti-CD8–RNP) were then washed three times with PBS buffer and then incubated with 1 M glycine (in PBS buffer) for 30 min with orbital agitation. The particles were washed with PBS (three times), resuspended in sterile PBS and kept at 4 °C until use. As a control, a model anti-goat antibody marked with FITC was also used for covalent coupling to PLGA particles following the procedure described above.

2.2.4. Maintenance of mammalian cell cultures

HEK293 (human embryonic kidney, ECACC No. 85120602) and TE671 (human Caucasian rhabdomyosarcoma, ECACC No. 89071904) cells were grown in D-MEM culture medium (Dulbecco's Modified Eagle Medium, Gibco) with 4.5 g/L glucose and GlutaMAX™ I and without pyruvate. CHO cells (Hamster Chinese Ovary, ECACC No. 85050302) were grown in F-12 medium with glutamine. All culture media were enriched with 10% foetal bovine serum and supplemented with 100 units/ml penicillin G sodium and 100 μg/ml streptomycin sulfate in 0.85% saline (GIBCO) to prevent for bacterial infections. Sub-culture was performed by trypsinization when cellular growth reached approximately 70% confluence. Cultures were grown in solid supports and kept at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air.

2.2.5. In vitro interaction assays of nanoparticles with mammalian cells

The affinity of anti-CD8 coated nanoparticles (anti-CD8–RNP) to CD8 expressing cells, via an antibody/antigen recognition system, was investigated by epifluorescence microscopy using CHO cells or CHO cells expressing CD8. To prevent cell culture contaminations, nanoparticles were resuspended and washed in sterile PBS supplemented with penicillin, streptomycin and fungisone (25 μg/ml). This suspension (60 mg/ml) was kept up to 2 weeks at 4 °C.

CHO cells expressing CD8 were obtained as follows: subconfluent cultures of CHO cells were transiently transfected with the pIRES-CMV-cCD8 construct by the FuGENE6 method according to the manufacturer's instructions. The construct contains the CMV promoter necessary for efficient expression in mammalian cells. Green fluorescence protein was co-expressed for positive selection of transfected cells. The transfection was performed with 1 μl cDNA:3 μl transfection reagent. To ensure that all cells showing GFP fluorescence also expressed the CD8 protein in the cell membrane we used Dynabeads (which are coated with the anti-human-CD8 antibody) as an additional detector for the presence of CD8 in the

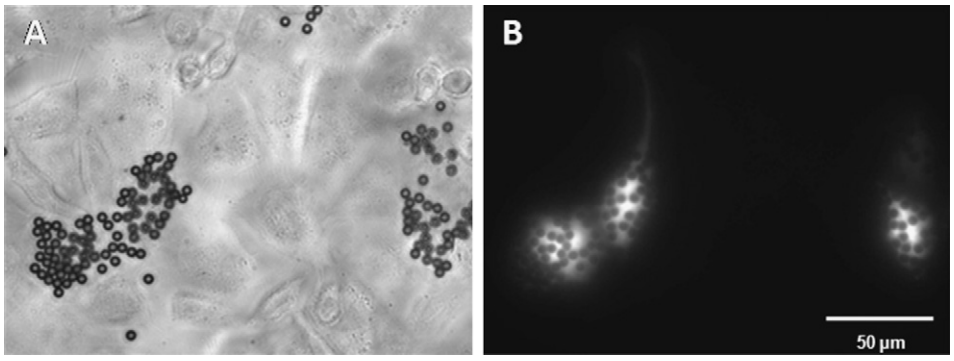

Fig. 1. (A) Phase contrast and (B) fluorescence microscopy images of CHO cells co-transfected with 1 μg cCD8 and 1 μg cGFP. After 48 h of transfection, all cells showing GFP fluorescence also express the CD8 protein in the plasma membrane (detected by the presence of Dynabeads at the surface of these cells). Therefore fluorescence due to GFP may be used as a marker for the presence of CD8.

membrane of CHO cells. In Fig. 1 it can be observed that after 48 h of transfection, all cells showing GFP fluorescence also express the CD8 protein in the plasma membrane (detected by the presence of Dynabeads at the surface of these cells).Therefore fluorescence due do GFP may be used as a marker for the presence of CD8.

The transfected cell culture was sub-cultured onto 13 mm diameter coverslips placed inside 35 mm diameter culture dishes filled with 3 ml of culture media. On the day of experiments, the culture media from the cell monolayers was replaced by 1 ml of solution of 0.5 mM EDTA in PBS. The EDTA was used to chelate the calcium ions necessary for cell adhesion and was removed by washing twice with PBS (3944 × g). The cells were resuspended in 1.5 ml of PBS to which 50 μl of anti-CD8–RNP suspension was added and incubated with gentle shaking for 1 h, at room temperature. Samples for observation under fluorescence microscopy were taken at different times. The recognition reaction was completed after 1 h of incubation.

To analyze non-specific interactions between cells and nanoparticles, controls were performed using unmodified NP containing rhodamine (RNP) using different cell lines. In order to assure a wide variety of cell properties three cell lines were chosen to perform these experiments namely HEK293, TE671 and CHO. Cell cultures were sub-cultured onto 13 mm diameter coverslips placed inside 35 mm diameter culture dishes filled with 3 ml of culture media. 50 μl (60 mg/ml) of RNP suspension were added to each dish and a coverslip with cells was removed for observation under the fluorescence microscope after 24, 48, 72 and 96 h. Prior to each observation, each coverslip was washed twice in PBS by gentle shaking for 5 min to remove excess of particles. The cultures grown in the presence of particles were kept as described previously.

All cell samples were observed with an Olympus Bx51 epifluorescence microscope under phase contrast optical microscopy with white light or with the fluorescence filters U-MWB2 (λ_{exc} = 460–490 nm, λ_{em} = 520 nm IF) or U-MWG2 (λ_{exc} = 510–550 nm, λ_{em} = 590 nm) to visualize the cells, the green fluorescent protein and the rhodamine, respectively. The images were monitored with Cell F-ViewImage System software.

3. Results and discussion

Nanoparticles containing rhodamine (RNP) were prepared from a PLGA polymer containing free carboxylic end groups using an emulsion solvent diffusion method. Rhodamine was used for a better visualization of nanoparticles. Similarly, PLGA nanoparticles (NP) were formulated without rhodamine for reference. The presence of the carboxylic acid groups on the particles surface would allow for covalent linkage to the NH_2 groups of the anti-human anti-CD8 antibody.

The surface morphology and size distribution of NP, RNP and anti-CD8–RNP were examined by scanning electron microscopy and representative images are shown in Fig. 2. The micrograph images revealed that the regular spherical shape and the smooth surface presented by the NP (Fig. 2A) are not affected by the presence of rhodamine or antibody (Fig. 2B and C) and that all produced particles have a size ranging from 350 to 600 nm. These values are in agreement with the sizes of 400 ± 100 and 550 ± 90 nm determined by laser scattering for RNP and anti-CD8–RNP, respectively. Compared to RNP, the anti-CD8–RNP size increased due to the presence of the antibody anti-CD8 on the particles surface.The zeta potentials of RNP and anti-CD8–RNP, measured by dynamic light scattering at neutral pH, were −0.96 ± 0.01 and −2.9 ± 0.2 mV, respectively, which indicates the presence of negatively charged surfaces. These values are lower than those previously reported for empty PLGA nanoparticles (near −25 mV). The presence of the anti-CD8 molecule, containing several ionisable groups, at the outer surface of nanoparticles affects, as expected, their superficial charge. Concerning the RNP, one would not anticipate such a decrease in the zeta potential. The decrease in the zeta potential may be explained by interactions established among rhodamine and polymer groups that affect the superficial charge of the nanoparticles. Nevertheless, in further studies performed by the group (data not shown), the produced particles have shown good stability also in aqueous phases for periods over 2 months.FTIR was used to evaluate the chemical structure of PLGA nanoparticles (Fig. 3A). The characteristic peak of PLGA at 1760 cm^{-1} due to the ester group, the bands at 3010 and 2955 cm^{-1} due to C–H stretch of CH_3, the band at 2885 cm^{-1} due to C–H stretch of CH_2, the peak at 1186–1089.6 cm^{-1} due to C–O stretch and the band between 3100 and 2800 cm^{-1} due to the carboxylic acid groups (Fig. 3A and B) were not altered in the NP and RNP spectra. The FTIR spectra of PLGA (data not shown) do not present any band at 3500–3100 cm^{-1}, characteristic of OH groups. Therefore, the visible band at 3500–3100 cm^{-1} (Fig. 3A and B) may indicate the presence of PVA on particles surface.

The presence of rhodamine was confirmed by fluorescence microscopy of the RNP (Fig. 4A) and the NP (Fig. 4C).

In order to verify the efficacy of the covalent linkage of the antibody to NP, coupling experiments were also performed using increasing concentrations of a model anti-goat antibody marked with the FITC fluorophore to yield fluorescent nanoparticles–antibody conjugates. The obtained bioconjugates were characterized by fluorescence microscopy (Fig. 5). An increase in fluorescence was obtained with an increase in the concentration of the antibody used and tended to reach saturation for concentrations higher than 20 μg/ml confirming the success of the coupling reaction. Also, the presence of CD8 onto RNP particles was con-

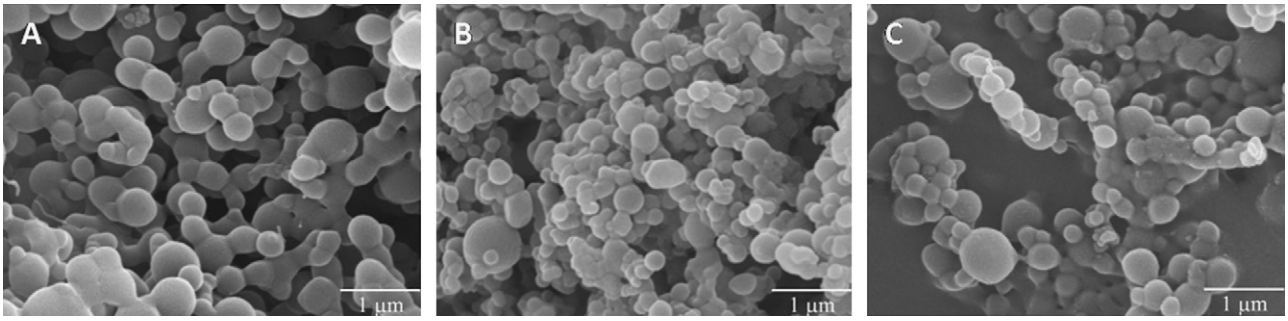

Fig. 2. SEM images of (A) PLGA nanoparticles (NP), (B) nanoparticles containing rhodamine (RNP) and (C) nanoparticles containing rhodamine and functionalized with the antibody anti-human CD8 (anti-CD8–RNP).

firmed by fluorescence microscopy of the anti-CD8–RNP (Fig. 6B) and the NP (Fig. 6C).

The anti-CD8 conjugated nanoparticles produced were tested for their ability to recognize target cells expressing the CD8 membrane protein at their surface, a recognized cellular marker of lymphoblastic leukaemia cells.

Our strategy was to transiently express the human CD8 membrane protein in CHO cell cultures and to perform recognition assays with particles to which the corresponding antibody was bound (anti-CD8–RNP). Transient transfection methods provided us the possibility to have cells expressing CD8 and non-expressing cells, which could be distinguished by the presence or absence of GFP, when evaluating the uptake of our delivery system. CHO transfected cultures were incubated with anti-CD8–RNP (Fig. 6A) and samples for microscopy observation were taken after 1, 2 and 4 h of contact time. It was observed that most of the recognition reaction had already occurred after the first hour. Fig. 6A shows a group of cells (left panel) where only two expressed the CD8 membrane protein (as detected by the GFP presence, middle panel). It is possible to observe that the anti-CD8–RNP bind only to the surface of these two cells (right panel). To assess the specificity of this binding, we have performed a similar experiment by incubating CHO transfected cultures with RNP and, up to 4 h of incubation, we have not been able to detect any cells presenting particles at their surface which indicates that there is no preferential binding of the particles to the cells expressing CD8 (Fig. 6B). These results demonstrated the preserved immunoreactivity of the anti-CD8–RNP and the specific interaction of the anti-CD8–RNP with the respective target cells.

The presence of the functionalized particles on the surface of target cells detected after only 1 h is in accordance with the results obtained by other groups (Cirstoiu-Hapca et al., 2007; Kocbek et al., 2007; Obermajer et al., 2007) using a monoclonal antibody specific for breast tumor cells (anti-cytokeratin) conjugated to PLGA nanoparticles to target cells (MCF-7 and MCF-10A neoT cells lines) (Kocbek et al., 2007) and anti-HER2 and anti-CD20 conjugated to PLA particles on SKOV-3 human ovarian cancer cells (overexpressing HER2) and on Daudi lymphoma cells (overexpressing CD20), respectively (Cirstoiu-Hapca et al., 2007). Scott et al. (2008) also observed a recognition reaction between antibodies specific to the siglec-7 (CD33-like) receptor conjugated to PLGA nanoparticles and target cells expressing CD33-like receptors, such as natural killer cells and monocytes. However, Dinauer et al. (2005) observed a recognition reaction only after 4 h when using the antibody anti-CD3 conjugated to gelatin nanoparticles surface to target cells expressing CD3 (Jurkat and CEM).

Our results show that RNP do not bind to CHO cells expressing CD8 up to 4 h of incubation. Similar results were obtained in other studies. However, these in vitro cell assays have been performed for short periods (4–6 h) (Cirstoiu-Hapca et al., 2007; Dinauer et al., 2005) whereas Kocbek and collaborators have performed more prolonged studies and showed that PLGA particles bind randomly

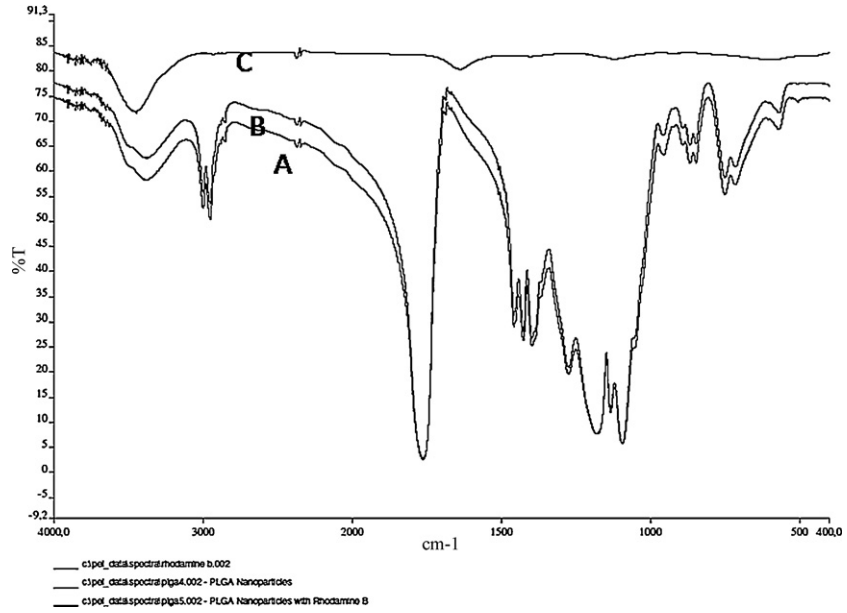

Fig. 3. FTIR spectra of (A) NP, (B) RNP and (C) rhodamine.

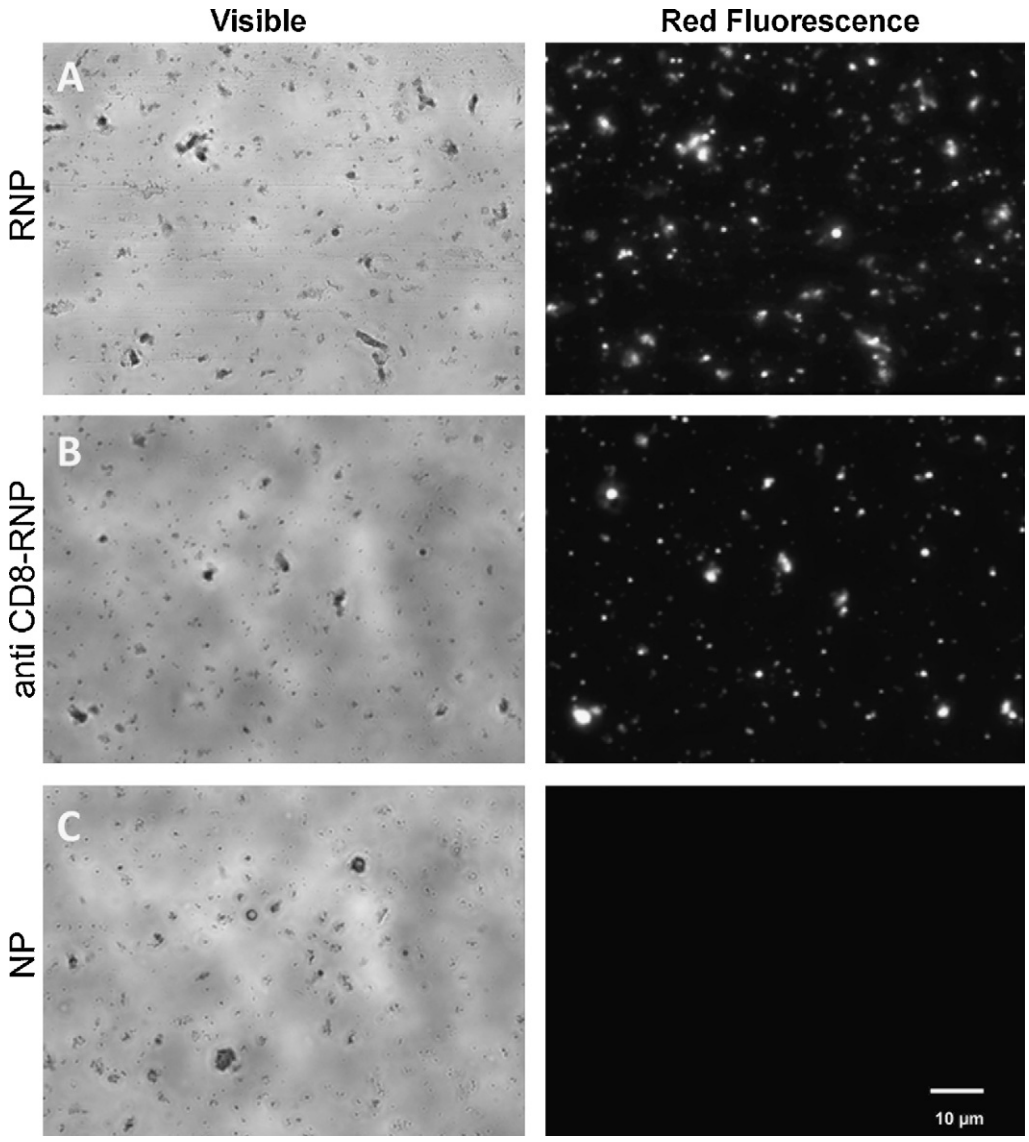

Fig. 4. Phase contrast (left) and fluorescence photographs (right) of particles (60 mg/ml) suspended in PBS (A) RNP, (B) anti-CD8–RNP and (C) NP (negative control).

on MCF-10A after 12 h and on Caco-2 cells after 24 h of incubation (Kocbek et al., 2007; Obermajer et al., 2007) and did not evaluate the possibility of nanoparticles internalization for longer incubation periods.

To test if the obtained results would not be specific of the cell culture and incubation time used we decided to investigate the possible internalization of RNP, per se, by other cell lines and for an incubation period longer than 72 h. We have grown three different mammalian cell lines: HEK 293, TE671 and CHO in the presence of RNP and followed the presence of particles by fluorescence microscopy at different incubation periods ranging from 24 up to 96 h. Representative results from each experimental condition are shown in Fig. 7. A general trend was observed for all cell lines, with an almost absence of particles on the cellular samples taken at 24 h of incubation and a growing number of cells showing particles with longer incubation periods. In parallel, the number of particles per cell also increased with the increasing incubation periods.

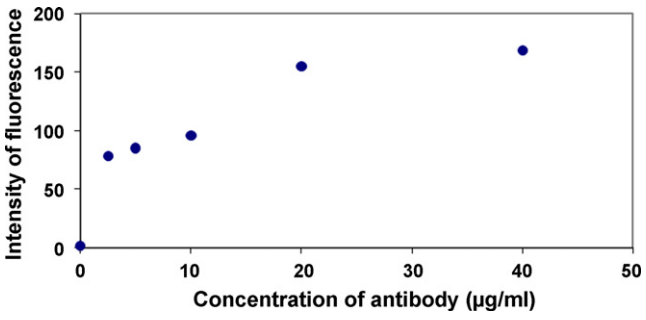

Fig. 5. Effect of antibody concentration on the fluorescence intensity observed on the antibody modified particles.

The present study shows that RNP have the ability to be internalized by mammalian cells after incubation periods longer than 24 h in accordance with the results obtained by Cartiera et al. (2009) for three types of epithelium cell lines. Interestingly, we observed slight differences in behaviour which seem to be characteristic of each cell line. We observed the presence of floating HEK 293 clusters of cells, an indication of cellular release from the coverslips onto which the culture was grown, which indicates a great sensitivity of these cells to the presence of particles.

In contrast, in the results obtained with anti-CD8–RNP (Fig. 6), particles were detected on cells surface in only 1 h. This fast and efficient binding of the anti-CD8–RNP to target cells may diminish

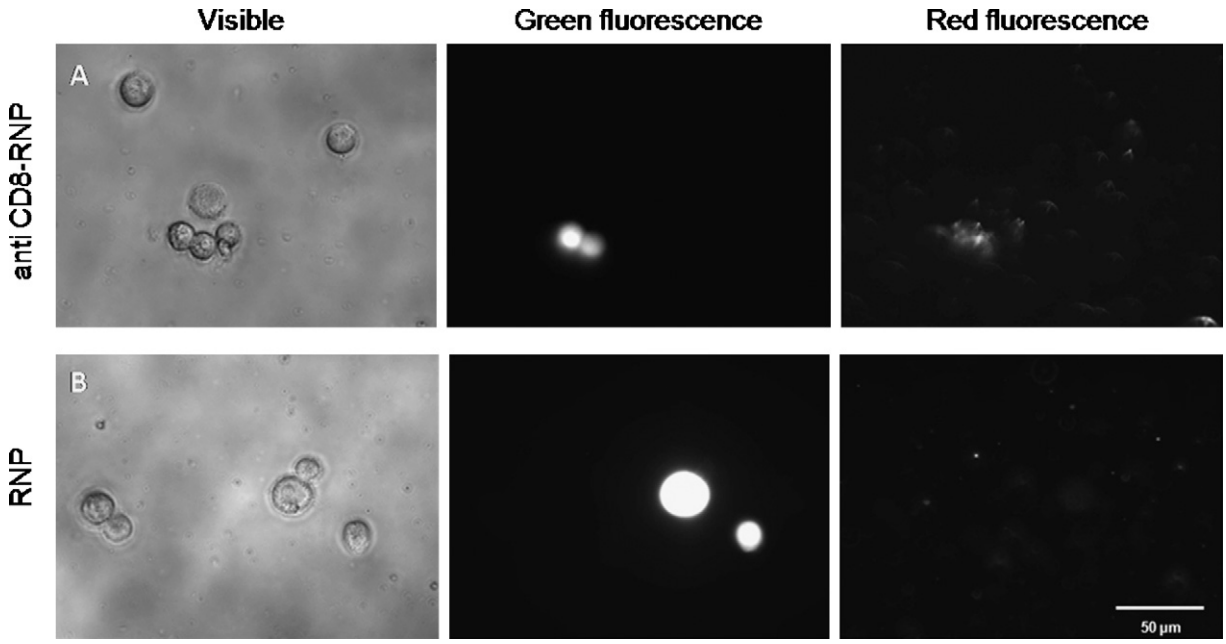

Fig. 6. Microscopy images of CHO cells expressing the CD8 membrane protein: (A) incubated with anti-CD8–RNP. The middle panel shows two cells expressing GFP, an indication of the presence of CD8 protein in the cells membrane. The right panel shows the potential binding of the marked particles to these cells. The reaction occurred after 1 h of incubation. (B) Incubated with RNP (negative control). There is no preferential binding of the particles to the cells expressing CD8.

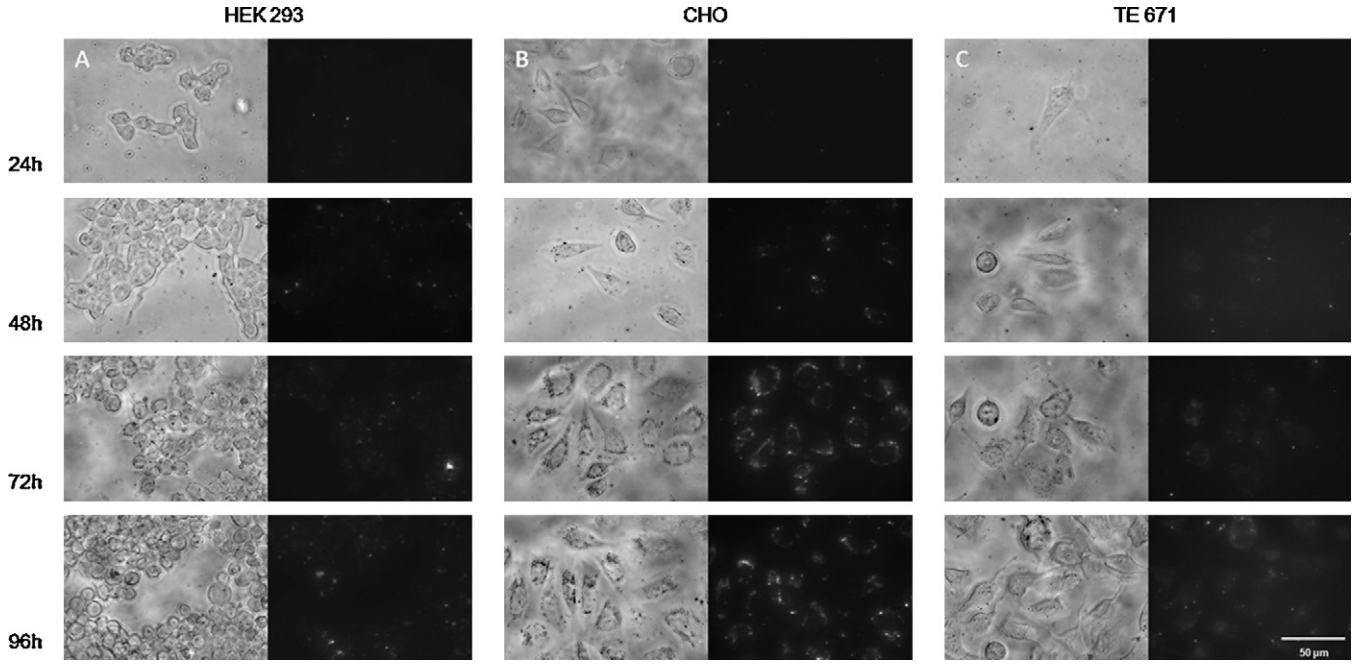

Fig. 7. Microscopy images of three different cell cultures (A) HEK 293, (B) CHO and (C) TE 671 incubated with RNP. Each set represents phase contrast (left panels) and fluorescence photographs (right panels) of cell samples for different incubation periods.

the unspecific interaction of the RNP with the cells that is observed for periods longer than 24 h. These results clearly indicate that the antibody anti-CD8 attached to the RNP surface show the ability to target cells expressing CD8.

Therefore, the developed nanoparticles could have the potential to selectively deliver therapeutics to sick cells. Our results suggest that this kind of approach may come to prove useful for in vivo control of leukaemia with the simultaneous minimization of unspecific side effects.

4. Conclusions

In vitro experiments in different cell lines show that, for long incubation periods (48 h), PLGA nanoparticles are randomly spread on cells surface. This fact can present a limitation in cancer therapy. In this study, we successfully synthesized PLGA nanoparticles covalently linked to the antibody against human CD8, a membrane protein which is a surface cellular marker of lymphoblastic leukaemia cells (Jurman et al., 1994; Pui and Jeha, 2007). The ability

of these nanoparticles to target cells expressing CD8 was seen from their selective distribution in a culture containing cells expressing CD8 and cells that do not express CD8. This recognition process was detected in 1 h and shows that the antibody maintains its specific recognition properties after covalent binding. The obtained results show that, in a shorter time, we can direct the available particles in suspension to target cells, which offers the ability to reduce side effects caused by unspecific drug uptake into healthy tissues. The CD8-nanoparticles produced represent a promising approach to improve the efficacy of nanoparticles active targeting for lymphoblastic leukaemia therapy.

Acknowledgments

Inês Peça is grateful for financial support from Fundação para a Ciência e a Tecnologia through contract SFRH/BD/48773/2008. The authors would like to acknowledge Dr. António Lopes (ITQB, UNL) for the transfer of knowledge related with the interpretation of DLS results and Dr. Paulo Lemos (REQUIMTE, FCT-UNL) for the help with the fluorescence microscopy.

References

Allen, T.M., 2002. Ligand-targeted therapeutics in anticancer therapy. Nat. Rev. Cancer 2, 750–763.

Bivas-Benita, M., Romeijn, S., Junginger, H.E., Borchard, G., 2004. PLGA-PEI nanoparticles for gene delivery to pulmonary epithelium. Eur. J. Pharm. Biopharm. 58, 1–6.

Brannon-Peppas, L., Blanchette, J.O., 2004. Nanoparticles and targeted systems for cancer therapy. Adv. Drug Deliv. Rev. 56, 1649–1659.

Cartiera, M.S., Johnson, K.M., Rajendran, V., Caplan, M.J., Saltzman, W.M., 2009. The uptake and intracellular fate of PLGA nanoparticles in epithelial cells. Biomaterials 30, 2790–2798.

Cegnar, M., Premzl, A., Zavasnik-Bergant, V., Kristl, J., Kos, J., 2004. Poly(lactide-co-glycolide) nanoparticles as a carrier system for delivering cysteine protease inhibitor cystatin into tumor cells. Exp. Cell. Res. 301, 223–231.

Cheng, J., Teply, B.A., Sherifi, I., Sung, J., Luther, G., Gu, F.X., Levy-Nissenbaum, E., Radovic-Moreno, A.F., Langer, R., Farokhzad, O.C., 2007. Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. Biomaterials 28, 869–876.

Cirstoiu-Hapca, A., Bossy-Nobs, L., Buchegger, F., Gurny, R., Delie, F., 2007. Differential tumor cell targeting of anti-HER2 (Herceptin) and anti-CD20 (Mabthera) coupled nanoparticles. Int. J. Pharm. 331, 190–196.

Clawson, C., Huang, C.-T., Futalan, D., Seible, D.M., Saenz, R., Larsson, M., Ma, W., Minev, B., Zhang, F., Ozkan, M., Ozkan, C., Esener, S., Messmer, D., 2010. Delivery of a peptide via poly(D,L-lactic-co-glycolic) acid nanoparticles enhances its dendritic cell-stimulatory capacity. Nanomedicine: NBM, Mar 27, 1–11.

Coester, C., Nayyar, P., Samuel, J., 2006. In vitro uptake of gelatin nanoparticles by murine dendritic cells and their intracellular localization. Eur. J. Pharm. Biopharm. 62, 306–314.

Csaba, N., Sánchez, A., Alonso, M.J., 2006. PLGA:poloxamer and PLGA:poloxamine blend nanostructures as carriers for nasal gene delivery. J. Control. Release 113, 164–172.

Davda, J., Labhasetwar, V., 2002. Characterization of nanoparticle uptake by endothelial cells. Int. J. Pharm. 233, 51–59.

Dinauer, N., Balthasar, S., Weber, C., Kreuter, J., Langer, K., Briesen, H.V., 2005. Selective targeting of antibody-conjugated nanoparticles to leukemic cells and primary T-lymphocytes. Biomaterials 26, 5898–5906.

Fahmy, T.M., Fong, P.M., Goyal, A., Saltzman, W.M., 2005a. Targeted for drug delivery. Nano Today 8, 18–26.

Fahmy, T.M., Samstein, R.M., Harness, C.C., Saltzman, W.M., 2005b. Surface modification of biodegradable polyesters with fatty acid conjugates for improved drug targeting. Biomaterials 26, 5727–5736.

Farokhzad, O.C., Cheng, J., Teply, B.A., Sherifi, I., Jon, S., Kantoff, P.W., Richie, J.P., Langer, R., 2006. Targeted nanoparticles–aptamer bioconjugates for cancer chemotherapy in vivo. PNAS 103, 6315–6320.

Farokhzad, O.C., Jon, S., Khademhosseini, A., Tran, T.-N.T., Van, D.A.L., Langer, R., 2004. Nanoparticles–aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res. 64, 7668–7672.

Hamdy, S., Molavi, O., Ma, Z., Haddadi, A., Alshamsan, A., Gobti, Z., Elhasi, S., Samuel, J., Lavasanifar, A., 2008. Co-delivery of cancer-associated antigen and Toll-like receptor 4 ligand in PLGA nanoparticles induces potent CD8+ T cell-mediated anti-tumor immunity. Vaccine 26, 5046–5057.

Jurman, M.E., Boland, L.M., Liu, Y., Yellen, G., 1994. Visual identification of individual transfected cells for electrophysiology using antibody-coated beads. Biotechniques 17, 876–881.

Kocbek, P., Obermajer, N., Cegnar, M., Kos, J., Kristl, J., 2007. Targeting cancer cells using PLGA nanoparticles surface modified with monoclonal antibody. J Control. Release 120, 18–26.

Li, Y.-P., Pei, Y.-Y., Zhang, X.-Y., Gu, Z.-H., Zhou, Z.-H., Yuan, W.-F., Zhou, J.-J., Zhu, J.-H., Gao, X.-J., 2001. PEGylated PLGA nanoparticles as protein carriers: synthesis, preparation and biodistribution in rats. J. Control. Release 71, 203–211.

Liu, Y., Miyoshi, H., Nakamura, M., 2007. Nanomedicine for drug delivery and imaging: a promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. Int. J. Cancer 120, 2527–2537.

Obermajer, N., Kocbek, P., Repnik, U., Kuznik, A., Cegnar, M., Kristl, J., Kos, J., 2007. Immunonanoparticles—an effective tool to impair harmful proteolysis in invasive breast tumor cells. FEBS J. 274, 4416–4427.

Panyam, J., Labhasetwar, V., 2003. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv. Drug Deliv. Rev. 55, 329–347.

Panyam, J., Sahoo, S.K., Prabha, S., Bargar, T., Labhasetwar, V., 2003. Fluorescence and electron microscopy probes for cellular and tissue uptake of poly(D,L-lactide-co-glycolide) nanoparticles. Int. J. Pharm. 262, 1–11.

Prabha, S., Zhou, W.-Z., Panyam, J., Labhasetwar, V., 2002. Size-dependency of nanoparticles-mediated gene transfection: studies with fractionated nanoparticles. Int. J. Pharm. 244, 105–115.

Pui, C.-H., Jeha, S., 2007. New therapeutic strategies for the treatment of acute lymphoblastic leukaemia. Nat. Rev. Drug Discov. 6, 149–165.

Sahoo, S.K., Labhasetwar, V., 2003. Nanotech approaches to drug delivery and imaging. Drug Discov. Today 8, 1112–1120.

Scott, C.J., Marouf, W.M., Quinn, D.J., Buick, R.J., Orr, S.J., Donnelly, R.F., McCarron, P.A., 2008. Immunocolloidal targeting of the endocytotic siglec-7 receptor using peripheral attachment of siglec-7 antibodies to poly(lactide-co-glycolide) nanoparticles. Pharm. Res.—Dordr. 25, 135–146.

Tobío, M., Gref, R., Sánchez, A., Langer, R., Alonso, M.J., 1998. Stealth PLA-PEG nanoparticles as protein carriers for nasal administration. Pharm. Res.—Dordr. 15, 270–275.

Yang, J., Lee, C.-H., Park, J., Seo, S., Lim, E.-K., Song, Y.J., Suh, J.-S., Yoon, H.-G., Huh, Y.-M., Haam, S., 2007. Antibody conjugated magnetic PLGA nanoparticles for diagnosis and treatment of breast cancer. J. Mater. Chem. 17, 2695–2699.