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decreased to 2276±288 nm at the end of 120 min having a polydispersity over 0.7 for both of the incubations.

Among the CLDC10 and CLDC11 prepared at the highest ratios (1:0.186 w/w) the complexation process was the fastest and the size observed between 2900 nm to 3044 nm remained nearly unchanged with CLDC10, whereas a progressive increase and then decrease was observed with CLDC11. The measured size of CLDC10 was 2412±62.7 nm at the end of 120 min, however the polydispersity index was over 0.7. The complexation was completed at all incubation periods for both complexes as confirmed by the gel electrophoresis analysis.

Conclusion

The results indicated that the incubation time required for complexation was variable. Maturation to form stable complexes is incubation time-dependent and needs to be optimized depending on the materials and experimental conditions used. These findings were consistent with the report of Yang and Huang [7] who described the time-dependent maturation and changes of the CLDC for serum resistance. Consequently, the DLS results of our study, which were also confirmed by gel electrophoresis, allowed to monitor the association and dissociation processes during complexation by an increase or decrease in the particle size of the CLDC. DLS provides a rapid tool for initial screening and selection of liposome/DNA complexes.

References

[1] Zelphati, O., Nguyen, C., Ferrari, M., Felgner, J., Tsai, Y., Felgner, P.L. (1998). Stable and monodisperse lipoplex formulations for gene delivery. Gene Ther., 5, 1272–1282.

[2] Eastman, S.J., Siegel, C., Tousignant, J., Smith, A.E., Cheng, S.H., Scheule, R.K. (1997). Biophysical characterization of cationic lipid:DNA complexes. Biochim. Biophys. Acta, 1325, 41–62.

[3] Lai, E., Zanten, van J.H. (2002). Real time monitoring of lipoplex molar mass, size and density. J. Control. Rel., 82, 149–158.

[4] Lee, L.K., Mount, C.N., Shamlou, P.A. (2001). Characterization of the physical stability of colloidal polycation–DNA complexes for gene therapy and DNA vaccines. Chem. Eng. Sci., 56, 3163–3172.

[5] Mount, C.N., Lee, L.K., Yasin, A., Scott, A., Fearn, T., Shamlou, P.A. (2003). The influence of physico-chemical and process conditions on the physical stability of plasmid DNA complexes using response surface methodology. Biotechnol. Appl. Biochem., 37, 225–234.

[6] Ross, P.C., Hui, S.W. (1999). Lipoplex size is a major determinant of *in vitro* lipofection efficiency. Gene Ther., 6, 651–659.

[7] Yang, J.-P., Huang, L. (1998). Time-dependent maturation of cationic liposome–DNA complex for serum resistance. Gene Ther., 5, 380–387.

[8] Rakhmanova, V.A., Pozharski, E.V., MacDonald, R.C. (2004). Mechanisms of lipoplex formation: dependence of biological properties of transfection complexes on formulation procedures. J. Memb. Biol., 200, 35–45.

[9] Pedroso de Lima, M.C., Neves, S., Filipe, A., Düzgüne°, N., Simoes, S. (2003). Cationic liposomes for gene delivery: from biophysics to biological applications. Curr. Med. Chem., 10, 1221–1231.

[10] Yargan, A.P. (2005). Investigation of the interactions of cationic liposomes and cationic liposome–DNA complexes with cell culture medium by particle size analysis and gel electrophoresis. MSc Thesis in Pharmaceutical Biotechnology Program, Hacettepe University, Health Science Institute, Ankara-Turkey.

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Microbubbles which bind and protect DNA against nucleases

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Summary

The aim of this study was to develop cationic microbubbles which can bind and protect plasmid DNA (pDNA). For this purpose, microbubbles were coated with poly-(allylamine HCl) (PAH) using the Layer-by-Layer technique.

Introduction

Ultrasound in combination with microbubbles (often called sonoporation) has recently acquired much attention in the field of gene delivery because of its low toxicity, simplicity and mainly because of its applicability *in vivo* without major complications. The mechanism by which ultrasound mediates cellular delivery has been ascribed as cavitation. Cavitation is the alternate growing and shrinking of formed or added gas-filled microbubbles as a result of the high and low pressure waves generated by the ultrasound. Eventually, these cavitating (oscillating) microbubbles can also implode due to these high pressure waves. The cavitation and especially the implosion of microbubbles generate local shock waves and microjets that can temporarily perforate the cell membrane and that can allow macromolecules to extravasate from the bloodstream. However,

a major limitation of the currently available microbubbles is that they have a short lifetime, and neither bind or protect the therapeutic DNA against nuclease. Binding of DNA to the microbubbles will assure that the DNA is present at the site of microbubble implosion, enhancing the chance that the DNA is dragged inside the cell or even the nucleus by the generated microjets. Consequently, the aim of this work was to develop a new type of ultrasound responsive microbubbles that can bind and protect the DNA against nucleases, and that stay stable during several hours.

Experimental methods

Preparation and characterization of uncoated and PAH-coated microbubbles

Microbubbles were prepared by mixing C_4F_{10} gas and an albumin–dextrose solution through a three-way-valve, followed by a short sonification. The PAH-coated microbubbles were prepared starting from the microbubbles above using the Layer-by-Layer (LbL) technique. Briefly, after washing the microbubbles were incubated with 5 mL of a PAH solution (2 mg/mL, hepes buffer). Subsequently, the PAH was removed by washing (three times) the microbubbles with sterile hepes buffer (20 mM, pH 7.4). Laser diffraction and zeta potential measurements were used to determine respectively the size distribution and the surface charge of the microbubbles.

DNA binding properties

Confocal laser scanning microscopy (CLSM) was used to visualize the binding of fluorescent labeled pDNA to the PAH-coated microbubbles. Picogreen was used to determine the maximal loading capacity of the coated microbubbles. Briefly, 150 μL of microbubble suspension was incubated with increasing amounts of pDNA and after 5 min of incubation time, the microbubbles were separated using centrifugation and the amount of pDNA in the subnatans was quantified using picogreen.

DNase I experiments

Microbubbles and pDNA were incubated for 5 min. Afterwards DNase I was added till a concentration of 200 U/L was obtained. After 15 min of incubation, microbubbles and pDNA were separated using a 5 M NaCl solution before centrifugation. Aliquots of the subnatans were run on a 1% agarose gel for 60–80 min.

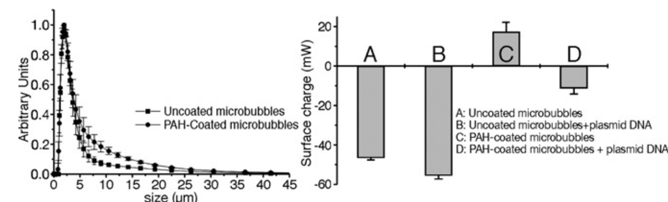

Fig. 1. Size distribution (left) and surface charge (right) of uncoated and PAH-coated microbubbles in the absence and presence of pDNA.

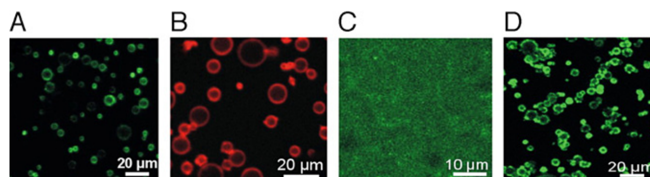

Fig. 2. CLSM images of (A) uncoated microbubbles prepared with FITC-BSA; (B) RITC-PAH-coated microbubbles prepared with unlabelled albumin; (C) unlabelled, uncoated microbubbles incubated with yoyo-1 labelled pDNA and (D) unlabelled, coated microbubbles incubated with yoyo-1 labelled pDNA.

Results and discussion

The size distributions and surface charges of the uncoated and polyallylamine hydrochloride (PAH)-coated microbubbles are shown in Fig. 1. About ~90% of the uncoated and coated microbubbles had a size between 1 and 5 μm. Microbubbles within such a size range are known to favour cavitation upon exposure to clinically used ultrasound frequencies (such as 1 MHz). Coating of the microbubbles with PAH, turned the surface charge positive (Fig. 1, right panel A–C), which may indicate that the microbubbles are indeed coated with PAH.

The albumin shell (FITC labelled, green) of the uncoated microbubbles and the PAH coat (RITC labelled, red) around these microbubbles was subsequently visualized using CSLM (Fig. 2A and B). The appearance of a red colored ring around the microbubbles further proved that the microbubbles are indeed coated with PAH.

Addition of pDNA to the uncoated microbubbles did not alter the microbubbles' surface charge. Incubation of uncoated microbubbles with yoyo-1 labelled pDNA (green) resulted in a rather homogeneous distribution of the green fluorescence (Fig. 2C). However, an accumulation of the pDNA around the microbubbles occurred when the PAH-coated microbubbles were incubated with the green labelled pDNA (Fig. 2D). Similar conclusions could be drawn from zeta potential measurements: addition of pDNA altered the ζ of the PAH-coated microbubbles from positive to negative (Fig. 1), which proves pDNA binding.

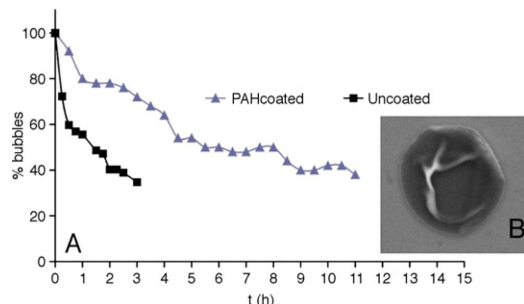

Fig. 3. Stability of uncoated and PAH-coated microbubbles. The insert shows a SEM-image of a PAH-coated microbubble after degradation.

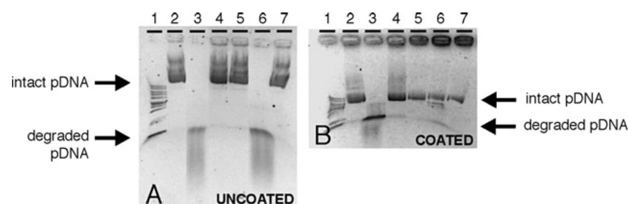

Fig. 4. Gel electrophoresis. Gels were run after NACL-induced dissociation of the pDNA. 'Smiling' appeared due to the high salt concentrations used (5 M NaCl). (A) Uncoated microbubbles; (B) PAH-coated microbubbles. Each lane contains 1 μg of plasmid DNA. (1) DNA molecular weight marker; (2) naked plasmid DNA; (3) naked plasmid DNA incubated with DNase I solution (200 IU/L); (4) plasmid DNA incubated with inhibited DNAse (200 IU/L); (5) microbubbles and plasmid DNA; (6) microbubbles and plasmid DNA incubated with DNase I (200 IU/L); (7) microbubbles and plasmid DNA incubated with inhibited DNase I (200 IU/L).

The picogreen assay revealed a maximum loading capacity of 0.1 pg/microbubble.

Perfluorocarbon gas-filled microbubbles are, despite the fact that they are covered by an albumin shell, very unstable. Indeed, the albumin shell does not completely prevent the diffusion of gas out of the microbubble. Coating of microbubbles with PAH could enhance their stability, A 5-fold increase of the half-life (from 75 min to 6 h) of the microbubbles was obtained after coating them with PAH (Fig. 3). The insert presents a SEM-image of a PAH-coated microbubble after degradation.

The ability of the microbubbles to protect pDNA against nuclease cleavage was tested using gel electrophoresis. The uncoated microbubbles were not able to protect pDNA from degradation by DNase I (lane 6 versus 3 of gel A). In contrast, the PAH-coated microbubbles were able to prevent degradation (lane 6 versus 2, 4 of gel B) Fig. 4.

Conclusions

Cationic charged microbubbles could be prepared by coating albumin stabilized microbubbles with PAH. These coated microbubbles had a 5-fold longer life and were capable of binding and protecting pDNA against nucleases. The maximal loading capacity of these coated microbubbles was estimated to be around 0.1 pg of pDNA/microbubble.

References

[1] Decher, G., Fuzzy nanoassemblies: Toward layered polymeric multicomposites, Science 277(5330) (1997) 1232–1237.

[2] Porter, T.R., Iversen, P.L., Li, S., Xie, F., Interaction of diagnostic ultrasound with synthetic oligonucleotide-labeled perfluorocarbon-exposed sonicated dextrose albumin microbubbles, J Ultrasound Med. 15(8) (1996) 577–584.

[3] Unger, E.C., Hersh, E., Vannan, M., McCreery, T., Gene delivery using ultrasound contrast agents, Echocardiography-A Journal of Cardiovascular Ultrasound and Allied Techniques 18 (4) (2001) 355–361.

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041

Novel ternary polyplex of triblock copolymer, pDNA and anionic dendrimer phthalocyanine for photochemical enhancement of transgene expression

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Summary

A novel ternary polyplex of triblock copolymer, pDNA and anionic dendrimer phthalocyanine was developed. The physicochemical characterization suggests the formation of tri-layered polyplex micelles with both pDNA and DPc incorporated in one compartment. The polyplex showed a 25 times photochemically enhanced transgene expression at optimum irradiation time compared to the non-irradiated control. Due to its neutral surface charge these polyplexes hold a promise for in vivo application after systemic delivery.

Introduction

One of the major requirements for in vivo gene therapy is development of a gene vector which can safely and effectively deliver a therapeutic gene into specific cells and can achieve regulated gene expression. The photochemical internalization (PCI) technology developed by Berg [1] offers light-inducible cytosolic delivery of the transgene. PCI is based upon the light activation of a photosensitizer specifically located at the membrane of endocytic vesicles inducing the rupture of this membrane upon illumination. Although this strategy allowed the light-induced transfection, the enhancement of gene expression was accompanied by remarkable photocytotoxicity. In the previous study, it appeared that photodamage towards sensitive organelles might be responsible for that photocytotoxicity. Also, there was discrepancy in the subcellular localization between pDNA polyplexes and photosensitizer that made the system less effective. Thus, the control of subcellular localization of photosensitizer might be a key factor in achieving light-induced transfection with minimal photocytotoxicity. Recently, a ternary complex composed of a core containing the packaged DNA and an envelope of anionic dendrimer phthalocyanine (DPc) has been developed in our laboratory. The ternary complex showed more than 100-fold photochemical enhancement of the transgene expression in vitro with reduced photocytotoxicity and demonstrated the first success of PCI-mediated gene delivery in vivo [2]. However this system is unlikely to be used for in vivo application after systemic delivery due to the negative surface charge. Thus