Gas Bubbles Stabilized by Multifunctional Nanoparticles for Ultrasound-Mediated Drug-Delivery

R. Schmid1, Y.A. Mørch1, P. Stenstad1, R. Hansen2, S. Berg2, Y. Hansen3, M. Afadzi3, S. Eggen3, H. Blom4 and C. de Lange5

1SINTEF Materials and Chemistry, 7465 Trondheim, Norway; 2SINTEF Technology and Society, 7465 Trondheim, Norway; 3The Norwegian University of Science and Technology, 7491 Trondheim, Norway, 4SciLifeLab, Stockholm, Sweden

ABSTRACT SUMMARY

A major obstacle in the delivery of nanoparticles (NPs) to tumor cells is the low uptake and heterogeneous distribution of the NPs in tumor tissue. Combining multifunctional NPs with focused ultrasound (US) treatment opens new possibilities in the combination of diagnosis and therapy and drug delivery mediated by a physical method. A novel multimodal, multifunctional drug delivery system consisting of microbubbles (MBs) stabilized by polymeric NPs has been developed. Stable PEGylated poly(butyl cyanoacrylate) (PBCA) (NPs) encapsulating fluorescent dyes, model drugs and MRI contrast agents were produced in a single step using a miniemulsion polymerization process. These NPs were further successfully used for stabilization of gas MBs with desired acoustic properties.

Initial in vivo studies in mice indicate a positive effect of US on NP extravasation and tissue penetration from these multifunctional MBs. Initial in vivo studies of the MBs in rabbits show high US contrast and longer circulation time than for commercial bubbles.

EXPERIMENTAL METHODS

Materials:

Unless otherwise stated, analytical grade chemicals were obtained from commercial sources and used as received. The monomer, n-butyl cyanoacrylate (BCA), was kindly provided by Henkel Loctite, Ireland.

Synthesis and characterization of multifunctional NPs:

NPs of the biocompatible and biodegradable polymer poly(butyl-2-cyanoacrylate) (PBCA) were synthesized using a miniemulsion polymerization process. Oil-in-water miniemulsions were prepared by emulsifying a monomer phase, consisting of butyl cyanoacrylate (BCA), 2% hexadecane or 3% Miglyol 810 as co-stabilizer and 0.05% fluorescent dye, in an acidic aqueous medium containing various types of surfactants by means of an ultrasonifier (Branson). The initiation of the anionic polymerization was carried out by adding various types of polyethylene glycols (PEGs) to the emulsion, resulting in PEGylated NPs.

Particle size, zetapotential and morphology were determined by dynamic light scattering, laser Doppler velocimetry (Malvern Zetasizer, Nano-ZS) and S(T)EM (Hitachi S-5500), respectively.

Synthesis and characterization of MBs:

MBs stabilized by PBCA NPs were prepared by mixing the nanoparticle dispersion with serum albumin and air using an ultra-turrax (IKA-Werke T25). The size and number of MBs was determined using Coulter Counter and their morphology was analyzed by fluorescence microscopy using both confocal laser scanning microscopy (CLSM, Zeiss LSM510 Meta) and structured illumination depletion (SIM, Zeiss Elyra PS). Ultrasound attenuation was measured using a setup described previously.

In vitro and in vivo studies of NPs and MBs:

Cells: PC3 (human prostatic carcinoma cell line).

Animals: Balb/c nude mice bearing a subcutaneous tumor (xenograft of human PC3 prostate adenocarcinoma) in the leg. Uptake of NPs in cells: The cellular uptake was quantified using flow cytometry, and CLSM was used to study the intracellular localization of the NPs. Distribution of NPs in tumor: Mice were injected intravenously (i.v.) with MBs stabilized with NPs or NPs alone and the tumors treated with focused US 5 min or 24 h later using different US exposures. The blood vessels were stained by i.v. injection of FITC labeled lectin 5 min
before sacrificing the mice. Tumors were frozen and microtomed, and sections analyzed by CLSM.

In vivo visualization of microbubbles:
Mice and rabbits were injected with PBCA NP-stabilized MBs while image acquisition using Vevo 2100 high frequency ultrasound imaging system from VisualSonics and GE Vivid E9 ultrasound system, for mice and rabbits respectively.

RESULTS AND DISCUSSION
Multifunctional nanoparticles:
Stable PEGylated PBCA NPs encapsulating fluorescent dye (as model substance) were produced in a single step using miniemulsion polymerization (Figure 1). The particle diameter could be varied from 100 to 250 nm (PDI<0.2) by varying the amount and type of surfactant and/or the sonification power. Various PEG-type initiators were tested for improved circulation time and biodistribution.

Microbubbles stabilized with multifunctional NPs:
MBs (diameter 1-6µm) stabilized by a shell of PBCA NPs were prepared by mixing the NP dispersion with serum albumin and air using an ultra-turrax (UT) (Figure 2). Imaging by CLSM and SIM demonstrated that there is a nanoparticle shell around the MBs consisting of 1-2 layers of NPs. The NP surface properties, especially the hydrophobicity/hydrophilicity, were found to be the most important factors determining the successful assembly of particles on the bubble surface. The stability of the MBs was dependent on the preparation parameters of the NP synthesis. Optimized batches showed superior stability compared to commercially available MBs with regard to US-exposure, and had very long shelf stability (> months).

The mechanical parameters and US resonance of NP-stabilized MBs were studied. The shape of the attenuation spectrum and the resonance frequency is dependent on bubble size, shell thickness and the mechanical properties of the shell. By varying the parameters in the preparation of the MBs, the resonance properties of the bubbles could reproducibly and predictably be varied over a range of frequencies covering those used in most diagnostic US.

In vitro and in vivo studies of NPs and MBs:
The cellular uptake of the NPs in vitro, depended on the length and type of PEG used.
In vivo, initial experiments injecting NP-stabilized MBs 5 min before US exposure into mice bearing tumors and visualized using an US imaging system for small animals demonstrated increased tumor uptake compared to animals not treated with US, suggesting enhanced extravasation. The MBs could easily be destroyed by increasing the ultrasound power output.
Injecting NPs alone and exposing the tumors to US 5 min or 24 h later, showed that US administered 5 min after NP-injection was more efficient. This indicates that the effect of US on extravasation was more important than the effect on penetration of NPs through the extracellular matrix. Furthermore US improved the distribution of NPs, i.e. the NPs were located further away from the blood vessels compared to untreated tumors where only small amounts of NPs were observed and located close to the blood vessels.
Initial in vivo studies of the MBs in rabbits showed high ultrasound (US) contrast and longer circulation time than for commercial bubbles.

CONCLUSION
Stable PEGylated PBCA NPs encapsulating fluorescent dyes, model drugs and MRI contrast agents were produced in a single step using a miniemulsion polymerization process. These NPs were further successfully used for stabilization of gas MBs with desired acoustic properties. The NP surface properties, especially the hydrophobicity/hydrophilicity, were found to be the most important factors determining the successful assembly of particles on the bubble surface.
Initial in vivo studies of the MBs in rabbits show high ultrasound (US) contrast and longer circulation time than for commercial bubbles. Initial in vivo studies in mice indicate a positive effect of US on nanoparticle extravasation and tissue penetration from these multifunctional MBs.

REFERENCES

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