Optimization and Characterization of PACA Nanoparticles for Drug Delivery and Imaging

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SUMMARY

Biodegradable PEGylated poly(alkyl cyanoacrylate) (PACA) nanoparticles (NPs) encapsulating fluorescent dyes and MRI contrast agents were produced in a single step using a miniemulsion polymerization process. The particles could also be conjugated with RGD peptide ligands. Whole animal imaging and LC-MS were found to be useful tools for determining the fate and concentration of NPs in vivo. Using PEG surfactants resulted in NPs with longer blood circulation time and these particles were found to be stable in all media tested. Further, the degradation profile of the PACA particles could be tailored by the choice of monomers used.

INTRODUCTION

The ability to create NPs incorporating multiple functionalities, including imaging and drug delivery as well as cell targeting, opens new possibilities in the combination of diagnosis and therapy. PACA NPs are promising candidates due to their easy and reproducible preparation, satisfying drug-loading capacity, biodegradability, low toxicity, and feasibility for production scale-up. To obtain sufficient accumulation at the disease site, the NPs need to stay in the circulation for a sufficiently long time. A common approach to achieve this is to coat their surface with poly(ethylene glycol) (PEG). In addition, the particle payload needs to be released in a controlled manner, according to the intended application. In the present study we show that PACA NPs can be designed with regard to surface functionalization, degradation and imaging properties.

EXPERIMENTAL METHODS

Synthesis of PACA NPs: PACA NPs were synthesized using a miniemulsion polymerization process. Oil-in-water miniemulsions were prepared by emulsifying a monomer phase, consisting of cyanoacrylate (BCA, IHCA or OCA), Miglyol®810 as co-stabilizer and near-infrared dye (DiR) or Fe@Fe₃O₄ (core@shell) particles, in an acidic aqueous medium containing either an anionic surfactant (sodium dodecyl sulphate, SDS) or a non-ionic PEG-based surfactant (Brij⁹L23, a polyoxyethylene lauryl ether) by means of an ultrasonifier. Anionic polymerization was initiated by adding a polyetheramine (Jeffamine®M-2070, PEG initiator) to the emulsion, resulting in PEGylated NPs. Non-PEGylated particles were made by initiation with NaOH. Particles were rinsed by dialysis and PEGylation was confirmed by ¹H-NMR.

Physicochemical characterization of NPs: Particle size, surface charge and concentration were determined by dynamic light scattering, laser Doppler velocimetry (both Malvern Zetasizer, Nano-ZS) and Nanoparticle Tracking Analysis (NanoSight) in various buffers and protein solutions. Particle morphology was determined by S(T)EM.

Degradation of NPs: Degradation kinetics of PACA particles was determined by tracking the NP concentration (NanoSight) or by Gas Chromatography by measuring the concentration of degradation products (alcohols). Pentanol was used as internal standard.

In vivo biodistribution and circulation studies: Animals: Balb/c nude mice with and without subcutaneous tumor (xenograft of human PC3 prostate adenocarcinoma) in the leg. Biodistribution of NPs: Mice were imaged using a Pearl Impulse small-animal imaging system while injected with DiR-containing NPs. DiR was excited at 785 nm and fluorescence detected at 820 nm with 85 µm image resolution. Mice were sacrificed and organs removed 24 h post injection. Accumulation of DiR in organs and tumors was determined both by imaging and LC-Mass Spectrometry. Circulation half-life: DiR fluorescence in blood samples from mice at various time points was measured by imaging 20 µl of plasma in the small-animal imaging system.

Targeting NPs: PACA NPs were functionalized with RGD targeting ligands by post-modification using streptavidin/biotin conjugation. Human umbilical vein endothelial cells (HUVEC) were incubated for 3 hours with RGD-NPs and NPs without RGD. The uptake of NPs in cells was determined using flow cytometry.

RESULTS AND DISCUSSION

Multifunctional nanoparticles: PEGylated PACA NPs encapsulating contrast agents for optical or MR imaging were produced in a single step (Fig 1). The NP size was 80-250 nm (PDI=0.2) and was controlled by the concentration of surfactant and sonication power.

Figure 1: S(T)EM image (left) and size distribution (right) of PEGylated PBCA NPs prepared in one step using the miniemulsion polymerization method.
LC-MS was found to be a powerful tool for quantification of NP payloads and polymer matrix composition and revealed almost 100% encapsulation efficiency of DiR. Fe@Fe$_3$O$_4$ particles (5-12 nm) were also successfully incorporated into the polymer matrix, allowing for the possibility of detection by MRI (Fig 2, ongoing studies).

Degradation of nanoparticles: At physiological conditions PACA NPs degrade by surface erosion. By varying the type of monomers or using mixtures of different monomers for NP production, the degradation rate could be controlled. Both the monomer type (Fig. 3A) and the pH (Fig. 3B) were found to significantly affect the degradation rate.

Protein binding and stability of NPs in various media: The formation of a protein corona on the particle surface may reflect the stealth efficiency of the PEG. Fig. 4 shows the change in NP size as a function of protein concentration. An increase in size was detected for non-PEGylated and SDS/PEG NPs, indicating a formation of protein corona on the NP surface. NPs with Brij/PEG were not significantly affected by the addition of proteins, indicating efficient PEGylation. Non-PEGylated and SDS/PEG particles were found to form aggregates in PBS and cell culture media, whereas Brij/PEG particles were stable under all conditions tested. Similar studies with RGD-modified NPs are ongoing.

Biodistribution and circulation half-life of NPs: Using near-infrared dye labeled NPs and whole animal scanning the fate of the various NPs was visualized. The circulation half-life of PACA NPs depends on the PEGylation strategy for particle production, and was found to be approximately 1.5 hrs when using SDS surfactant and approximately 2 hrs when using PEG-based surfactant (Brij). NPs were found mostly in liver and spleen, but SDS/PEG NPs accumulated faster in these organs compared to Brij/PEG NPs.

Targeting NPs: PACA NPs conjugated with biomarkers for specific receptors were produced. Whereas only 13-22% of HUVEC had taken up PEGylated NPs, 97-98% of the cells had taken up PEGylated PACA additionally conjugated with RGD. The biodistribution and targeting ability of these NPs with biomarkers is currently being tested in vivo in our laboratories.

CONCLUSION We have shown that PACA NPs can be designed with regard to surface functionalization, degradation and imaging properties, and can hence be tailored for various drug delivery and imaging applications.

REFERENCES

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