Polymer-based colloidal nanoparticles as contrast agents for Magnetic Resonance Imaging (MRI) with the potential of drug delivery systems

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Objective

Nanoparticles (NPs) were prepared by both classical oil-in-water (o/w) emulsion method and water-in-oil in water (w/o/w) solvent evaporation technique, and were designed to serve as contrast agents for magnetic resonance imaging (MRI) and to have the potential to mediate drug delivery. Several crucial parameters including the gadolinium (Gd) and protein content of the nanoparticles, their size and dispersity were determined. Magnetic resonance measurements and imaging were carried out by intravenous perfusion of mono-disperse suspensions of the nanoparticles into human vital blood vessels (vessels of placental cotyledons) and into rats.

Methods

Double emulsion nanoparticles were prepared according to a modified [(water-in-oil) in water] solvent evaporation technique (1)-(3). The inner aqueous phase (W1), containing 2.8 % polyvinyl alcohol (PVA, polymer 1) and an MR contrast agent (gadopentetate dimeglumine, Magnevist) solution was emulsified in 1 % polylactic acid (PLA, polymer 2) in dichloromethane (O) with Ultra Turrax IKA DI 25 basic. This primary emulsion, containing PVA and MR contrast agent was then reemulsified in an outer aqueous phase (W2) with a lectin (*lycopersicon esculentum agglutinin*, LEA) as a blood vessel targeting group. The single emulsion PLA-HSA-DTPA-Gd NPs comprises HSA-DTPA-Gd (human serum albumin-gadolinium conjugate) as signal enhancer in the outer protein corona layer. The synthesized nanoparticles were designed to serve as carriers of Gd chelates and specifically bind to blood vessels. Experimental parameters were varied during nanoparticle preparation (including the nature and concentration of the surfactant, homogenizer speed, lyophilization) to elucidate their effects on the MR properties of the nanoparticles and to optimize the preparation. NPs were characterized by different spectroscopies, size exclusion chromatography, Bradford protein assay, PCS (photon correlation spectroscopy), TEM and SEM (transmission and scanning electron microscopy) for assessment of NPs' morphology and integrity.

A) Double Emulsion Nanoparticles: W1/O/W2

Emulsify 2 ml of PVA (polyvinyl alcohol) 2.8 % (w/v), containig 0.5 ml Magnevist (0.5 M) in 12 ml CH_2Cl_2, containing 1 % PLA at 9500 rpm.

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Primary emulsion: W1/O

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Reemulsify in 109 ml 0.4 % (w/v) PVA, containing 8.00 mg LEA at a) 8 000 or b) 20 500 rpm for 3 min.

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Double emulsion: W1/O/W2

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Stir over night at RT.

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Collect nanospheres by centrifugation at 3 345 x g, 15 min.

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Resuspend nanoparticles in buffer or liophilize.

B) Poly-(D,L-lactides)-HSA-Gd:

1) Preparation of HSA-DTPA-Gd conjugate:

DTPABA suspended in DMSO was added stepwise to a HSA solution in HEPES buffer (100 mM, pH 8.8). Adjust pH to 8.5 by means of 3 M NaOH. Stir for 2 h at RT .

↓

Dialysis I with cut off 12 400 Da.

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Complexation with Gd

↓

Dialysis II (with cut off 12 400 Da) and lyophilization

2) PLA-HSA-Gd nanoparticles:

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Emulsify PLA, solved in CH_2Cl_2 into 2% w/v HSA-Gd in H_2O at 24 000 rpm for 3 min.

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Stir over night at RT.

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Dialysis with cut off 100 000 Da or high speed centrifugation at 3345 x g for 15 min.

Figure 1. Synthesis flow schemes for the production of DE W1/O/W2 and PLA-HSA-Gd nanoparticles.

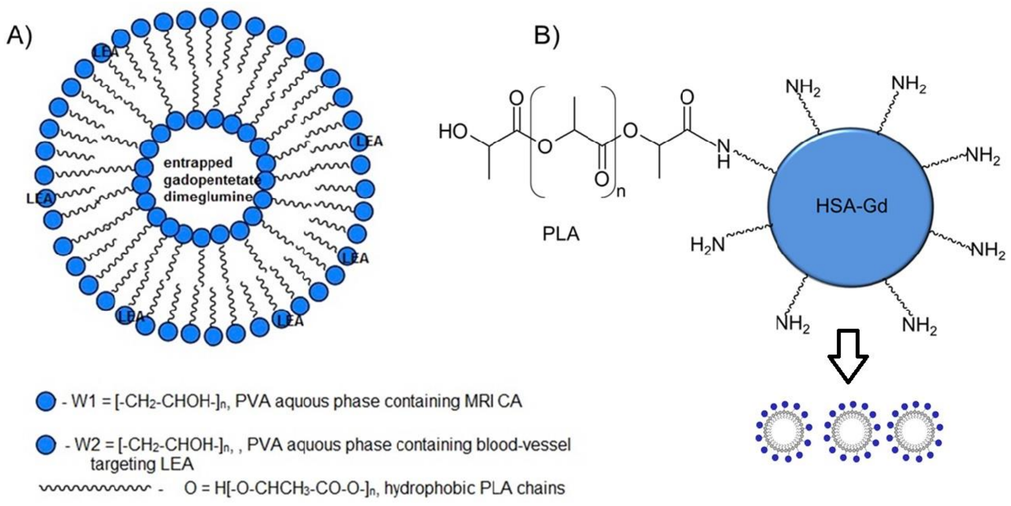

Figure 2. Proposed structures of A) DE W1/O/W2 and B) PLA-HSA-Gd nanoparticles.

Results

Monodisperse NPs' emulsions, carrying DTPA-Gd or HSA-DTPA-Gd were synthesized by emulsification-solvent evaporation. The prepared constructs showed uniform size of 194 nm (peak area 64%) for PLA-HSA, average Z-Ave 509 nm (peak area 63%) for W/O/W variant a) and 328 nm (peak area 54%) for variant b). The Gd amount in the nanoparticles has been determined by inductively coupled plasma mass spectroscopy (ICP-MS) and lay between 0.13-0.63 mM. Contrast-rich MR imaging was obtained, showing that polymer based nanoparticles can be used as MRI contrast agents.

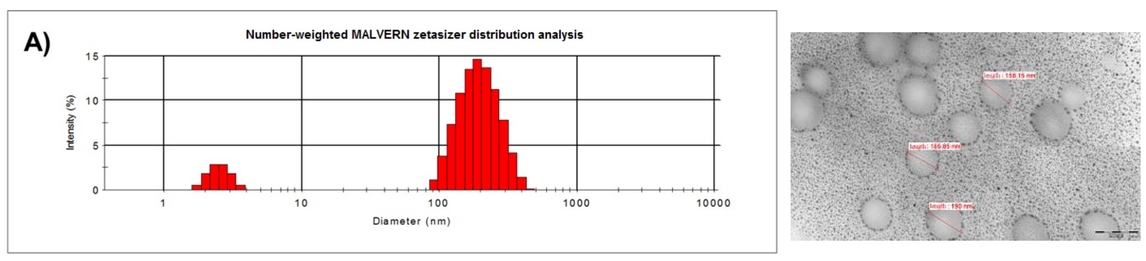

Figure 3. PCS of A) PLA-HSA-Gd nanoparticles: average size is 64 % 194 nm with corresponding TEM image right; B) and C) Double emulsion nanoparticles variant a) 63 % with 509 nm and variant b) 54 % with 328 nm and 31 % with 18 nm respectively. To the right corresponding SEM images.

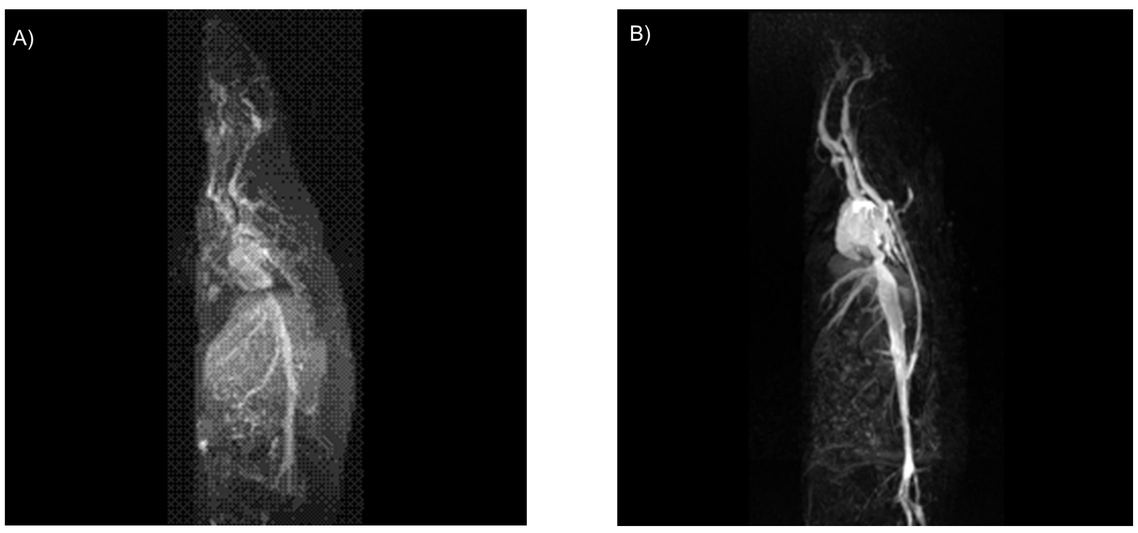

Figure 4. Angiogram-like MRI images of PLA-HSA-Gd nanoparticles in rat: A) 1 min and B) 4 min after contrast agent's application.

Conclusion

Double and single emulsion PLA-based nanoparticles were successfully prepared and characterized. The stabilized core-shell structure and biodegradable biopolymer composition makes them potential application candidates for controlled delivery of bioactive compounds such as anticancer drugs and as biospecific diagnostic tool in MRI. Advantages can be taken by overcoming the poor water solubility of many drugs by incorporation into the NPs' lipophilic layers. Thus opening new perspectives for use of NPs' emulsions for selective molecular imaging and cancer therapy simultaneously - theranostic concept.

References: (1) Montisci M J et al, International Journal of Pharmaceutics 215, Issues 1-2 , 2001, Pages 153-161; (2) Lai P et al, Colloids and surfaces B, Biointerfaces 118, 2014, 154–163; Pashkunova-Martic I et al, Mol Imaging Biol 13(1), 2011, 16-24.