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Biocompatible nanoparticles and gadolinium complexes for MRI applications

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ABSTRACT

Performances of double-emulsion techniques (W/O/W and W/O/O) and ionotropic gelation process were compared to achieve encapsulation of gadolinium MRI contrast agents (GdCAs) into biocompatible polymeric nanoparticles (NPs) with high Gd-loadings. The better approach proved to be ionotropic gelation with H[Gd(DOTA)] as GdCA. Relaxometry evaluation of H[Gd(DOTA)]⊂NPs efficiency demonstrated that incorporation of H[Gd(DOTA)] inside an hydrogel matrix highly improved H[Gd(DOTA)] relaxivity. Particle efficacy as MR contrast agents was further demonstrated on a 3 T clinical imager: a significant improvement of T_1- and T_2- MR signals was obtained at doses much lower than the currently used.

RÉSUMÉ

L'encapsulation d'agents de contraste IRM à base de gadolinium (GdCAs) dans des nanoparticules (NPs) polymères biocompatibles a été réalisée par double émulsion (W/O/W et W/O/O) et gélation ionique. La gélation ionique s'avère la meilleure stratégie en présence de H[Gd(DOTA)]. L'évaluation relaxométrique des H[Gd(DOTA)]⊂NPs montre que l'incorporation de cet agent dans une matrice hydrogel exalte sa relaxivité. L'efficacité de ces NPs comme agents de contraste pour l'IRM est mise en évidence sur un imageur clinique 3 T : une amélioration significative des signaux en modes T_1 et T_2 est observée pour des doses bien plus faibles que ce qui est actuellement administré.

1. Introduction

Magnetic resonance imaging (MRI) is a routinely used non-invasive diagnostic tool providing high-resolution anatomical images (sub-millimeter spatial resolution). Physical principles of MRI rely on the relaxation of water protons, which depends on the magnetic fields (the strong static magnetic field B_0 and the radiofrequency field), on the pulse sequence, and on the heterogeneous distribution and environment of water in the organism [1]. Indeed, variations of proton longitudinal (T_1) and transversal (T_2) magnetic relaxation times are partly responsible for the image contrast [1]. However, in some examinations, the

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information obtained from a simple MR image is not sufficient to highlight areas of interest. Thus, the contrast has to be improved, by administering optimal contrast-enhancing agents (CAs). The most currently used contrast agents are T_1-CAs, constituted of paramagnetic complexes of metal ions with symmetrical electronic ground states, such as gadolinium (GdCAs) [1,2]. Paramagnetic T_1-CAs predominantly shorten the longitudinal relaxation times of water protons in their vicinity. This effect gives rise to signal intensity increase (positive CAs) and then to a better differentiation between healthy and pathological tissues. GdCA efficiency is defined by its relaxivity r_i ($mM^{-1}\ s^{-1}$), which corresponds to selective reduction of the relaxation times of the water protons brought about by a 1 mM concentration of paramagnetic centers. To improve this efficacy, the Solomon-Bloembergen-Morgan theory [3] establishes outlines to design more efficient contrast agents [4]. For applications at 0.5–1.5 T, relaxivity improvement per Gd center can be achieved by high Gd-loadings and by controlling the tumbling motion of the GdCAs, through association of metal complexes to macro- or supramolecular carriers [5]. Another way to insure reduced tumbling rates, and larger payloads of active magnetic centers, is to graft or to encapsulate them into nanoparticles (NPs). Different nanoplatforms such as viral capsules, lipoprotein or apoferritin, [5] as well as liposomal NPs, [2b] micelles, [2b] Nanoscale Metal-Organic-Frameworks, [6] fullerenes [7] and inorganic NPs [8] have been used. However, the biocompatibility of these objects is a crucial factor whenever *in vivo* applications are implied. In this respect, polymeric biocompatible NPs are ideal candidates for the vectorization of contrast agents.

To improve MRI GdCA efficiency, our purpose was then to encapsulate some currently marketed contrast agents into a biocompatible matrix, by using a fully biocompatible protocol. Since GdCAs are highly hydrophilic chelates, the challenge was to achieve high Gd-loadings inside the NP framework. Recently, we have demonstrated that hydrophilic copper complexes, homologous to GdDOTA, can be satisfactorily incorporated in PLGA (Poly[D, L-lactide-co-glycolide]) nanoparticles through a double-emulsion technique [9]. In this paper, we demonstrate, *via* several adaptations of the double-emulsion technique, that this methodology is not appropriate for GdCAs. We also compare these attempts to an alternative protocol recently published, based on a ionotropic gelation process which involves hydrophilic biopolymers [10]. For each protocol, encapsulation of a set of GdCAs was attempted. Systematic evaluation of NP morphologies, GdCA encapsulation efficiencies, as well as NP production yields allowed to select the best combination between polymer matrix and Gd contrast agents. For the optimal condition, relaxation rate measurements ($1/T_1$ and $1/T_2$) at 20 and 60 MHz were performed.

2. Experimental

2.1. General

All chemicals were used as received without further purification. Solvents were of pharmaceutical grade. Poly(D, L-lactide-co-glycolide) (PLGA, 50:50 lactide/glycolide) was purchased from Sigma–Aldrich, France. Sodium hyaluronate was extracted from *Streptococcus equi* sp. (Sigma-Aldrich, France). Chitosan (low-molecular weight) was purchased from Sigma (France). Poloxamer 188 (Pluronic® F-68, polyethyleneglycol-co-polypropyleneglycol-copolyethyleneglycol) was purchased from Sigma-Aldrich, France. Lipoid® S75 was purchased from Lipoid GMBH (Germany). Stelliesters® was kindly donated by Stéarinierie Dubois (France). 1,2,3-Triacetoxypropane (triacetin) was purchased from Sigma–Aldrich, France. Ninety-five percent (v/v) ethanol was obtained from Charbonneaux Brabant (France). Sterile water for injections (Aqua B. Braun, Melsungen, Germany) was systematically used for nanoparticle preparation and analysis.

Gd-CAs used in this study were commercially available (Dotarem®, Guerbet, France; Multihance®, Bracco Imaging, France; Prohance®, Bracco Imaging, France; Magnevist®, Bayer Santé, France).

2.2. Nanoparticle syntheses

2.2.1. W/O/W double-emulsion protocol

2.2.1.1. Basic protocol. The formation of Gd-CA loaded nanoparticles was achieved by adjusting the multiple emulsion technique, *i.e.* water (internal, Wi)-in-oil (O)-in water (external, We) previously described for Cu-complex encapsulation [9]. Briefly, 750 μL of aqueous triacetin-saturated phase Wi consisting of the chosen Gd-CAs ($\sim 5 \times 10^{-5}$ mol) and Poloxamer P188 0.1% w/v, pH = 10 was emulsified in an organic phase O of PLGA 3% w/v in 5 mL triacetin, under ultrasonic stirring (Vibracel VC 750, ~ 20–25 °C, 30 s, 22%). Then, 10 mL of an aqueous triacetin-saturated solution containing the same percentage of Poloxamer P188 were added to this primary emulsion to obtain the double-emulsion (Wi/O/We). Afterwards, the droplets were converted into nanoparticles through the solvent diffusion step, carried out with addition of 150 mL of ethanol. Raw nanoparticle suspensions were obtained within 0.5 h.

2.2.1.2. Alternative strategy 1. An alternative protocol was used by replacing Poloxamer P188 by Tween® 20, 5% w/v in all aqueous phases, while maintaining all other parameters identical.

2.2.1.3. Alternative strategy 2. A second alternate strategy was used by replacing the organic phase O by one containing PLGA dissolved in triacetin, added with various amounts of Poloxamer P188 (0.75, 1.5 or 3% w/v). All other parameters were kept constant.

2.2.2. W/O/O double-emulsion protocol

Gd-CA loaded nanoparticles could also be prepared by a totally biocompatible multiple emulsion technique water (W)-in-oil (O_1)-in-oil (O_2). Briefly, 750 μL of aqueous triacetin-saturated phase Wi consisting of the chosen Gd-CAs ($\sim 5 \times 10^{-5}$ mol) and Tween® 20, 5% w/v, pH = 10 was emulsified in an organic phase O of PLGA 3% w/v in 5 mL triacetin, under ultrasonic stirring (Vibracel VC 750, ~ 20–25 °C, 30 s, 22%). Then, 10 mL of an organic solution O_2