Paclitaxel-induced formation of 3D nanocrystal superlattices within injectable protein-based hybrid nanoparticles†

Jeong Yu Lee, Ho Yeon Son, Jae Chul Park, Jongnam Park and Yoon Sung Nam a,b,c,†

Clusters of superparamagnetic iron oxide nanocrystals (IONCs) have been studied as contrast agents for magnetic resonance imaging (MRI) to visualize specific molecules, cells, and tissues, and to trace the transport routes of drug and gene delivery carriers. When used for these purposes, the IONCs are encapsulated within or coated by organic (e.g., polymers, lipids, sugars, and surfactants) or inorganic (e.g., silica) materials, which help maintain their dispersion stability in biological media. Clustering of multiple IONCs within a nanoparticle has been proven to increase transverse relaxivity (r2), which is directly related to detection sensitivity in MRI. Until now, however, no studies have reported on the impact of a close-packed assembly of IONC superparticles within a colloidal dispersion on r2 and MRI sensitivity.

Here we report on the linker-induced self-assembly of monodisperse IONCs into a 3D superlattice using paclitaxel as a linker molecule within cross-linked protein-based nanoparticles (Fig. 1). The nanoparticles were prepared via the simple emulsification of an organic solution mixture of IONCs and amine-reactive six-arm-branched polyethylene glycol (PEG) in an aqueous solution containing human serum albumin (HSA). The addition of paclitaxel induced the formation of a close-packed superparticle structure of IONCs, with a diameter of about 150 nm. We also investigated the effects of the formation of 3D superlattices on signal enhancement in MRI, and the biological activities of paclitaxel incorporated within the IONC superlattice.

Fig. 1 Schematic representation of paclitaxel and oleic acid of IONC assembly (i) and encapsulation with paclitaxel in cross-linked HSA-PEG NCs (ii).
The monodisperse IONCs were synthesized according to previously reported procedures. Their average diameter was 11.7 ± 0.4 nm, and the surface was stabilized with oleates. Organic droplets containing a mixture of the IONCs (5 or 10 mg mL⁻¹) and amine-reactive six-arm-branched PEG in dichloromethane (11.3 mg mL⁻¹) were generated in an aqueous solution of HSA (1 mg mL⁻¹), followed by cross-linking of the protein and PEG at the organic/aqueous interface. As the organic solvent diffused out of the droplets and was evaporated, the cross-linked HSA/PEG was shrunk, and IONCs were successfully entrapped within the HSA/PEG nanocapsules (denoted 'IONC-HSA/PEG NCs') with an encapsulation efficiency of ~100%. Transmission electron microscopy (TEM) images indicated that the IONCs were loosely aggregated within the nanocapsules (Fig. 2a–c). Unexpectedly, when paclitaxel (5 mg mL⁻¹) was added to the organic solution containing IONCs (5 mg mL⁻¹) before emulsification in a protein solution (1 mg mL⁻¹), the IONCs self-assembled into a close-packed 3D superlattice in the core region of the HSA/PEG hybrid nanoparticles (Fig. 2e–g). We presumed that paclitaxel induces strong surface affinity between the IONCs via hydrophobic and van der Waals interactions, resulting in a molecular bridge between the IONCs due to its hydrophobic and planar structure. Although several methods of preparing colloidal superparticles have been reported in recent years, they require elaborate steps and long processing time. In contrast, our method takes just a few minutes to build the superlattice structure using a simple emulsification process. More importantly, the resulting IONC-PTX-HSA/PEG NCs are readily dispersible and highly stable in an aqueous serum solution without the aid of surfactants, which is highly desirable for biological applications.

The average diameter of the IONC superlattice in the HSA/PEG NCs was 159.1 ± 46.1 nm when the overall size of nanocapsules was 298.5 ± 58.2 nm as determined from TEM images (n = 30). The average rim-to-rim distance between the adjacent IONCs was about 24.4 nm, and these measurements correspond to an average interparticle gap of about 1.1 nm. The measured value is smaller than the double thickness of a contracted oleic acid monolayer (1.7 nm). This result indicates that some ligands between the adjacent IONCs can be replaced by paclitaxels, which can attract the IONCs via pi–pi interactions. The size of the IONCs in the HSA/PEG NCs was appreciably affected by the concentration of IONCs in the oil phase. When the concentration of paclitaxel was increased from 5 mg mL⁻¹ to 10 mg mL⁻¹ (3.7 to 8.2 mg mL⁻¹ loaded) the IONC-PTX-HSA/PEG exhibited a relatively dense-packed IONC superlattice, and the average diameter of the superlattices increased from 159.1 nm to 180.2 nm.

To determine whether or not other multi-ring aromatic molecules can induce the ordering of IONCs with the HSA/PEG NCs, we tested 9,10-dimethylanthracene (DMA), pyrene, and deoxycholic acid as the linker molecules. Among the three molecules, DMA stably induced a cross-fringe pattern of IONCs (Fig. S4, ESI†). Under high resolution, the IONC structure was observed to be hexagonal. In some cases, the isostructural lattice of IONCs coexisted in the core of the HSA/PEG NC by changing their direction in the same plane projections. However, pyrene, another multi-ring aromatic hydrocarbon, did not show the effect under the same conditions (Fig. S5, ESI†), and the most hydrophobic bile acid, deoxycholic acid, induced a compact IONC structure without any patterns (Fig. S6, ESI†). These results indicate that only particular aromatic additives can induce the formation of non-close packed single-component superlattices of IONCs. The precise underlying mechanism of the formation of 3D superlattice is not clear yet, but the molecular feature of paclitaxel and DMA suggests that pi–pi stacking can significantly contribute to the self-assembly of IONCs within the protein nanocapsules. To further demonstrate these structural differences, we obtained fast Fourier transform (FFT) patterns, shown in Fig. 2d, h, and l. The FFT patterns, taking the form of optical diffractograms of selected areas in the TEM images of IONC-HSA/PEG and IONC-PTX-HSA/PEG, provided structural information from the electron diffraction patterns of the areas in superlattices. For the IONC superlattice loaded with paclitaxel in HSA/PEG NCs, the dots were in alignment, and the symmetry of each layer was confirmed by the FFT pattern. The XRD profile of IONC-PTX-HSA/PEG also indicates the superstructure peak arising at (001) reflection (2θ = 9.5°) (Fig. S7, ESI†).

Magnetic measurements of the IONC superlattice were carried out using nuclear magnetic resonance (NMR) and magnetic
resonance imaging (MRI) (Fig. 3). Relaxivity, $r_2$, is proportional to $M^2d^3$, where $M$ is the magnetization value of IONCs and $d$ is the diameter of the IONCs, and thus high $r_2$ can be achieved by using materials with large particles with strong magnetization value.\(^{28}\) The MR contrast effects of the IONC superlattice were compared with those of the IONC-HSA/PEG NCs at the same iron concentration. The IONC-HSA/PEG NCs showed relatively weak MR contrast with $r_2$ of 273.0 mM$^{-1}$/s$^{-1}$ (Fig. 3a). In contrast, the $r_2$ of the IONC-PTX-HSA/PEG NCs was 520.2 mM$^{-1}$/s$^{-1}$ for the same condition, which was almost twice higher than that of the IONC-HSA/PEG NCs. The superior $r_2$ of the IONC-PTX-HSA/PEG NCs was further verified by comparative MR images in a concentration-dependent manner at an iron concentration of 0 to 3.6 mM (Fig. 3b). The $T_2$-weighted MR images of the IONC-PTX-HSA/PEG NCs showed instantaneous enhanced negative contrast at an iron concentration of 0.11 mM, while the IONC-HSA/PEG NCs could be detected above an iron concentration of 0.23 mM. A commercially available MRI contrasting agent, Resovist,$^{25}$ was used as a control sample with the same iron concentrations and showed insensitive changes in MR contrast at the given concentrations. This remarkable improvement in NMR detection and MR imaging can be explained by the synergistic magnetism induced by the high density of IONCs in the core of the HSA/PEG NCs. The assemblies of magnetic particles can lead to a concomitant decrease in the spin–spin relaxation time ($T_2$) of adjacent water protons.$^{29,30}$ Following incubation with IONC-PTX-HSA/PEG NCs, PC3 cells showed a markedly increased negative signal on $T_2$-weighted MRIs compared with IONC-HSA/PEG (Fig. 3c). Consequently, the decreased relaxation time increased the relativity of the IONC-PTX-HSA/PEG, even with the same IONC concentration.

Paclitaxel is a well-known anti-cancer drug, and its therapeutic potential for cancer treatment is beyond question. In this study, paclitaxel is thought to be functioning as a sort of ligand between IONCs. To evaluate whether this phenomenon can affect the therapeutic activity of paclitaxel, we examined different formulations of nanoparticles using two different cancer cell lines (Fig. 4). HeLa cells (human epithelial carcinoma cells) and MCF 7 cells (human breast cancer cells) were chosen as model cancer cells because of their responsiveness to paclitaxel. The cytotoxicity of PTX-HSA/PEG, IONC-HSA/PEG, IONC-PTX-HSA/PEG, and a mixture of PTX-HSA/PEG and IONC-HSA/PEG were determined at 48 h post-treatment. Cell viability was assayed quantitatively using a cell-counting kit-8 (CCK-8) assay, in which the formation of formazan dye depends on the activity of the mitochondria.$^{31}$
When the cells were exposed to high concentrations of PTX-HSA/PEG, IONC-PTX-HSA/PEG, and a mixture of PTX-HSA/PEG and IONC-HSA/PEG, both types of cells responded almost identically as a function of paclitaxel concentration. 165 μM. Loss of cell viability (93.9%) was significantly notable in the MCF 7 cells exposed to 50 μM of paclitaxel within the IONC-PTX-HSA/PEG, and the HeLa cells also exhibited a 91.1% decrease at the same concentration. Overall, the viabilities of IONC-PTX-HSA/PEG between 0.1 and 50 μM were almost the same as PTX-HSA/PEG. The results indicate that the anti-cancer activity of paclitaxel loaded within the superlattice was preserved, and thus IONC-PTX-HSA/PEG can be used for theranostic applications.

In conclusion, we demonstrated a new method of generating a nanocrystal superlattice of IONCs within protein–polymer nanocapsules. The presence of multi-ring aromatic additives such as paclitaxel or dimethylnaphthacene induced the self-assembly of IONCs, leading to the formation of a superlattice structure. The concentrated structure produced by the self-assembly of IONCs in the protein nanocapsules showed potential for enhanced magnetic resonance. By including a guest-molecule, we have shown for the first time that an anti-cancer drug can induce the three-dimensional assembly of a nanocrystal superlattice. More importantly, the paclitaxel used in this study efficiently suppressed the proliferation of HeLa cells and MCF 7 cells without degrading the distinctive structure. This new finding is expected to be employed to provide simultaneous enhanced biomedical diagnosis and cancer therapy.

Conflicts of interest

There are no conflicts to declare.

References