MILK PROTEIN COATED IRON OXIDE NANOPARTICLES FOR MAGNETIC RESONANCE IMAGING

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Purpose

As natural products, proteins are intensively studied and much effort has been made on the development of protein-based nanotheranostics. They have unique structural and physicochemical properties which could facilitate their functionalities, such as high affinity to bind ions or small molecules, pH-responsive properties etc. In addition, they also have excellent biocompatibility and biodegradability, which is essential for the biomedical applications. In the previous studies, transferrin, apoferritin, human serum albumin have been adsorbed onto the surface of the nanoparticles to form functionalized entirety for the development of molecular and cellular imaging probes.[1-3] These examples generated significant interests in developing protein coated nanoparticulate imaging probes and drug delivery platforms, fabricating protein based nanoparticles as well as studying the interactions between proteins and inorganic nanoparticles.[4-5] Casein (CS) is a main ingredient of milk, which is a family of related phosophoproteins (e.g. for bovine casein, including α-, β-, κ-caseins). Naturally, casein exists as micelles acting as nanovehicle for delivery of calcium, phosphate and bioactives. In this study, we fabricated water-soluble casein coated iron oxide nanoparticles (CSIOs) through the ligand exchange and subsequent encapsulation. Resulting CSIOs showed improved transverse relaxivity over conventional polymer coating and has excellent water solubility, rending a promising new class of MRI contrast agents.

Method

The hydrophobic iron oxide nanoparticles (IOs) are prepared from heating iron oxide powder with oleic acid.[6] After exchanging the oleic acid molecular with oligosaccharides on the surface, the particles (SIOs) were then encapsulated into casein to form protein coated iron oxide nanoparticles (CSIOs). TEM, DLS, Gel electrophoresis, IR, UV-vis, Bradford assays are used to characterize the product. For evaluating MRI contrast, the phantom solutions of CSIO with different concentrations were examined using a 3T MRI scanner using T1 and T2 weighted fast spin echo sequences and multi-echo T2 weighted spin echo sequence, which allowed for obtaining transverse relaxation times. The scan parameters include: repetition time (TR) is 2000 ms, and the echo time (TE) is started at 10 ms with increments of 10 ms. Iron oxide nanoparticles in the same core size coated with amphiphilic triblock co-polymer reported previously (SHP15) was used for comparing the MRI contrast enhancement and the effect of different coating materials.[6,7] The signal intensities (SI) of each ROI (region-of-interest) from multi-TE images were measured for each concentration, and T2 relaxation times was calculated from the measured average SI using a non-linear exponential curve fitting over different TEs. To test the stability at different pH, the CSIOs solution was adjusted into the desired pH with HCl or NaOH. Subsequently experiments for investigating pH-dependent changes of DLS, Zeta potential measurements, MRI contrast and the transverse relaxivity were performed.

Results

The TEM results shows that the nanoparticulate core remained unchanged after coating with CS. The hydrodynamic size of CSIOs increased as the result of the CS coating on IOs. Gel electrophoresis also shows the increased size of CSIOs, and confirmed the presence of protein coating. The protein coating (white cage) around iron oxide nanoparticles can be visualized by TEM with negative staining. CSIOs exhibit strong T2 weighted contrast in T2 weighted MRI phantom studies. The transverse relaxivity R2 of CSIOs is measured as 272.85 $s^{-1}mM^{-1}$. Comparing with the IOs coated with conventional polymers (R2 = 109.04 $s^{-1}mM^{-1}$), CSIOs demonstrated imporved MRI contrast with a similar size and surface charge. Further, CSIO has a fairly stable MRI contrast effect over a wide range of pH. Though the hydrodynamic size and surface charge change with pH values, the R2 value did not change significant except at low pH below pI (~4.0).

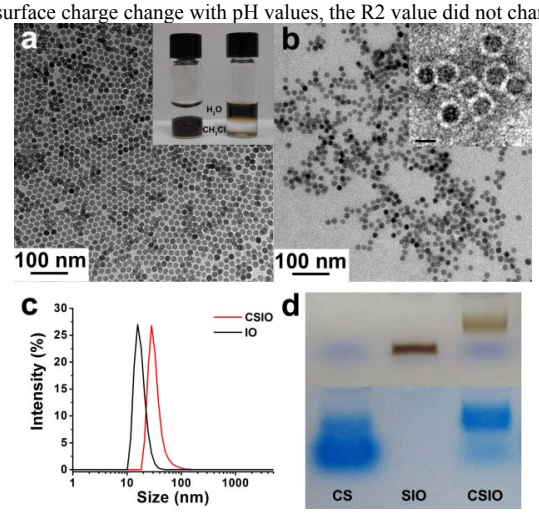

Fig 1. a) TEM images of IOs before modification, inset is colour photographs of hydrophobic IOs dispersed in chloroform (right) and water-soluble CSIOs (left); b) TEM images of CSIOs, inset is negative staining TEM images showing protein coating (white); c) DLS data showing the change of hydrodynamic size of IOs before and after modified with casein; d) photograghs of gel eletrophoresis analysis of casein (CS), iron oxide nanoparticles after surface ligand exchange with oligosaccharide (SIO), casein coated iron oxide nanoparticles (CSIOs) (top), and gel code blue staining (bottom) to visualize protein (blue band).

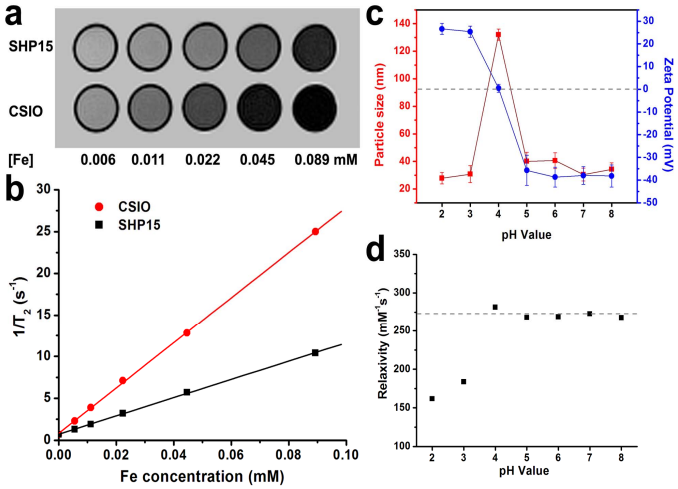

Fig 2. a) T2 weighted spin echo MR images of CSIOs and SHP15 at different Fe concentrations; b) T2 relaxation rates (1/T2, s^{-1}) as a function of Fe concentration (mM), the R2 value is calculated by non-linear exponential curve fitting to be 272.85 $s^{-1}mM^{-1}$ and 109.04 $s^{-1}mM^{-1}$ for CSIOs and SHP15, respectively; c) plots of CSIOs hydrodynamic size change (red) and the surface charge change (blue) as a function of pH, the isoelectric point pI of CSIOs is determined to be ~4.0; d) transverse relaxivity R2 as a function of pH, the R2 value did not change significantly at the pH range of 4-8, but decrease sharply below the pI.

Conclusions

A milk protein coated iron oxide nanoparticles (CSIOs) have been successfully synthesized through the ligand exchange and subsequent encapsulation. The protein coating has been verified through the TEM, DLS, UV and Gel electrophoresis. These CSIOs exhibits higher R2 relaxivity, which is improved over the same nanoparticles coated with conventional polymers. The high R2 relaxivity is believed to be contributed to the specific protein coating. As a result of successful coating with casein, the CSIOs also show pH-responsive properties with pI~4.0.

References:

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