Ultra-small Multimodal (Fluorescent and Paramagnetic) Chitosan Nanoparticles

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Introduction

Multimodal nanoparticles (MNPs) are a new class of engineered imaging probes that received immense interest in recent years for non-invasive *in vivo* imaging such as tracking of stem cells [1] and cancer imaging [2]. Detailed understanding of stem cell function demands development of highly-sensitive MNPs that would allow tracking of a few stem cells in the presence of millions of other cells. It is desirable to develop MNPs that are ultra-small (< 20 nm) and biocompatible. Multimodal MNPs that are fluorescent and paramagnetic [ref] are attractive as the combination of these two imaging modalities could potentially provide significant information at the single cell level. It is however challenging to fabricate ultra-small size "biologically-friendly" MNPs with bright fluorescence and superior paramagnetic properties. In this report, we present a robust method of fabricating ultra-small (<20 nm) uniform size fluorescent and paramagnetic MNPs that are biodegradable and targetable. Using water-in-oil (W/O) microemulsion technique, biodegradable chitosan based MNPs (CMNPs) were synthesized where fluorescein isothiocyanate (FITC, a fluorescent molecule) and DOTA-Gd (a stable Gd(III) chelating complex that is paramagnetic) served as multimodal image contrast agents.

Experimental

A mixture of appropriate amounts of FITC labeled and DOTA-Gd labeled chitosan polymer was dissolved in Triton X-100/n-hexanol/cyclohexane/water W/O microemulsion. The microemulsion water droplet served as nanoreactor for the synthesis of CMNPs. When appropriately cross-linked, CMNPs retain their spherical integrity and allowed easy separation and purification from the microemulsion. Particle size was measured by the transmission electron microscopy (TEM). Fluorescence excitation and emission spectra of the CMNPs was recorded using a fluorescence spectrometer (SPEX, Horiba Jobin Yvon). Paramagnetic properties of the CMNPs were characterized using 4.7T Bruker magnet (a NHMFL facility at UF-AMRIS). Particle surface charge was determined by zeta potential measurements. These CMNPs were hydrophilic and highly-soluble in phosphate buffer saline (pH 7.4).

Results and Discussion

The average particle size was ~18 nm as measured by the TEM (**Figure 1**). The CMNPs showed strong fluorescence and paramagnetic properties. Upon excitation at 490 nm, strong fluorescence at 520 nm (characteristic to FITC dyes) was recorded. Bright green emission was visualized when exposed to a 366 nm multiband hand-held UV excitation source. The CMNPs were stable for several months under refrigeration. MRI measurements in solution showed bright contrast against control (**Figure 2**; shown contrast against water as well as the effect of serial dilution of the sample, Ch1 through Ch5). Proton relaxivity values, R_1 and R_2 were calculated to be 54 $mMGd^{-1}s^{-1}$ and 75 $mMGd^{-1}s^{-1}$, respectively. The zeta potential value was measured to be ~ +20 mV.

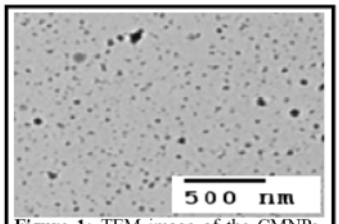

Figure 1: TEM image of the CMNPs. The average particle size (based on 50 particles) was calculated to be ~ 18 nm.

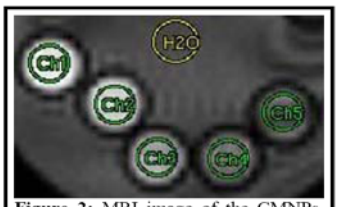

Figure 2: MRI image of the CMNPs. The MR contrast against serial dilution of the sample (Ch1 - most concentrated, Ch5 most diluted) as well as water (control).

Conclusions

Ultra-small (<20 nm), monodispersed biodegradable CMNPs were synthesized using W/O microemulsion method. These CMNPs showed strong fluorescence and MRI properties. Particles were highly dispersed in aqueous medium. It is expected that higher loading efficiency of CMNPs could result from ultra-small size and mild cationic surface properties. Further evaluation of the CMMPs under in vitro and in vivo conditions is underway.

Acknowledgements

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References

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[2] Kircher, M.F., *et al*., Cancer Research, **63(23)**, 8122-8125 (2003).